Physiological aspects of stomatal regulation and water use in wheat (*Triticum aestivum* L.) under terminal drought

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Abstract

Wheat grown in Mediterranean-type environments is often exposed to end-of-season drought (terminal drought). As terminal drought develops, soil dries in the top layers of the soil profile, exposing the upper part of the root system to soil water deficit while roots below may still have access to deeper soil water. It is hypothesised that the part of the root system exposed to drying soil induces abscisic acid (ABA) production and the corresponding increase in leaf ABA concentration partially closes stomata, which regulates the use of available water at depth, allowing completion of grain filling. The aim of this research was to examine if ABA accumulation and regulation of stomatal conductance in response to drying soil, would improve water use and grain yield under conditions of terminal drought. Four experiments were conducted from 2011 to 2014 in controlled environmental conditions.

In the first experiment described in Chapter 3, four wheat genotypes were grown in a split-root system and terminal drought was induced in half of the vertically-split root system, while the other half was kept well watered. Genotypes were selected for their putative adaptation to contrasting dryland environments in Australia. The aim of this experiment was to identify genotypes with contrasting stomatal responses and leaf ABA concentration. The two most contrasting genotypes identified were the cultivar Drysdale and the breeding line IGW-3262. When one half of the root system was exposed to drying soil, leaf water potential decreased and Drysdale had lower stomatal conductance and higher leaf ABA concentration. Leaf water potential remained unchanged in IGW-3262 but stomatal conductance decreased without concomitant increase in leaf ABA concentration.

In the second experiment (Chapter 4), Drysdale and IGW-3262 were grown in segmented pots that hydraulically isolated top and bottom root segments. Withholding water from the top segment alone or from both segments at anthesis exposed either the top half of the root
system or the entire root system to terminal drought. Drysdale had more root biomass in the top drying segment and initiated stomatal closure earlier than IGW-3262. There was a strong curvilinear relationship between leaf ABA and stomatal conductance in Drysdale, but the relationship was weak in IGW-3262. Leaf ABA in IGW-3262 fluctuated regardless of watering treatments which suggests that factors other than soil water deficit control leaf ABA. The increase in leaf ABA in water-stressed plants preceded a decline in stomatal conductance; probably xylem ABA is the driving factor for stomatal regulation. The source of xylem ABA was not identified as increased root ABA was not detected in either genotypes in response to drought. Despite sufficient water availability in the bottom segment, yield in IGW-3262 was similar to plants where both segments were dried, but Drysdale had higher yield. The results suggest that Drysdale and IGW-3262 differ in their stomatal response to ABA and in their root capabilities to capture available water at depth.

In the third experiment (Chapter 5), a transpiration assay was conducted to determine (i) whether xylem ABA is the main driver of stomatal regulation and (ii) whether the two genotypes differ in their sensitivity to (a) xylem ABA concentration, (b) xylem sap pH or (c) soil water status. Exogenous ABA was fed to detached leaves to identify the stomatal response to xylem ABA. The assay confirmed that IGW-3262 stomata are sensitive to factors other than leaf ABA concentration such as pH and possibly osmolality, whereas Drysdale stomata are relatively insensitive to these factors.

In the fourth experiment (Chapter 6), post-anthesis water use of Drysdale and IGW-3262 was compared under conditions where water was available in the bottom 30 cm layer of the 1 m soil profile. It was hypothesised that Drysdale used more water which results in less yield gap under terminal drought compared with IGW-3262. The grain yield of Drysdale under terminal drought was similar to that under well-watered conditions. However, grain yield in IGW-3262 decreased by 25% despite available water in the bottom layers of the soil profile. The yield reduction in IGW-3262 corresponded to the reduction in post-
anthesis water use. The difference in post-anthesis water use when the top soil layer was dried but water was available at the bottom was due to differences in root capability to capture water. This difference may be associated with differences in root anatomy or hydraulic properties.

In summary, this research shows that grain yield under terminal drought depends on post-anthesis water use regulated by stomatal closure and root capability to extract available water from soil. The cultivar Drysdale, which was more sensitive to soil water deficit and had better root capability to extract water from deeper soil, maintained water uptake during grain filling, resulting in better yield under terminal drought. This study also demonstrates that the wheat genotypes, Drysdale and IGW-3262, differ in their stomatal sensitivity to soil water deficit, leaf ABA concentration and pH. Future studies involving more genotypes are required to identify genotypic variation in stomatal regulation and sensitivity to ABA that provide an advantage under terminal drought through more effective water use. Understanding the link between root properties and stomatal regulation that facilitate post-anthesis water use and yield maintenance are important for developing improved wheat cultivars capable of maintaining yield under terminal drought.
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Figure 6.7 Adaxial (a,b) and abaxial (c,d) stomatal conductance (g_s) in IGW-3262 and Drysdale when drought was induced from anthesis by withholding water completely (WS), withholding watering to 60% pot water capacity and then watering was restricted to the bottom 30 cm of the soil profile (WB) and when watering maintained pot water holding capacity at 90% from anthesis to physiological maturity (WW). Each value is the mean of four replicates. Error bars are SEM. .........................................................119
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Statement of candidate contribution

The work presented in this thesis does not contain any material that has been accepted for award of any other degree or diploma at any other University.

1. I declare that this thesis is my own work and was not written by another person except where acknowledgement is made in the text. This thesis contains material previously published in the journal: ‘Field Crops Research’ 2014, volume 162.

2. The above publication pertaining to this thesis was written by me and the co-authors were involved in the discussion of results, structure of the paper and editorial comments to finalise them. All contributions made by other individuals have been duly acknowledged.

3. I declare that I received assistance from Dr Everard Edwards for ABA analysis of Chapters 3, 4 and 5.

Renu Saradadevi
1 General introduction

Wheat (*Triticum aestivum* L.) is the third most produced cereal after maize and rice in the world (FAO, 2013). Global wheat production averages 650 million metric tonnes (Mt), 65% of which is used as food (FAO, 2013). In Australia, more than half of the cropping area is used for wheat production with 40% of the wheat produced coming from southern and western Australia (ABARES, 2013). This wheat-growing region has a Mediterranean-type climate characterised by wet winters and dry summers. High variability in rainfall and temperatures in this area leads to frequent end-of-season drought (terminal drought) (Turner and Begg, 1981). Predictions for further reductions in rainfall and increased temperature (Ragab and Prudhomme, 2002) are forecasting more frequent drought spells (Farre and Foster, 2010), that threaten future grain production in the south-western Australian cropping region. Simulation studies have forecast yield reduction of more than 80% under severe terminal drought in this region (Asseng et al., 2004). Considering the current pressure on the agricultural production system to meet increasing global demands for wheat, it is crucial to focus research on reducing the gap between potential yield and actual yield under drought scenarios.

When terminal drought develops, soil dries from the top, exposing the shallow part of the root system to dry soil while the bottom part is in contact with available soil water. Plant roots sense the drying soil and produce signals, which on transmission to shoots trigger stomatal closure to regulate the crop water use through transpiration (Gollan et al., 1986; Blum and Johnson, 1993). The plant hormone abscisic acid (ABA) is considered the major chemical signal involved in this process and there is evidence of increased ABA accumulation in leaves of drying wheat plants (Henson et al., 1989b; Ali et al., 1998). However, crop production is linked to crop transpiration (Sinclair et al., 1984) such that limiting transpiration may reduce yield.
The role of ABA in regulating stomatal aperture in response to drought has been mainly studied on crops other than wheat such as maize, sunflower and tomato. Studies conducted on wheat have been limited and mainly focussed on seedlings and plants during the vegetative growth (Munns and King, 1988; Munns, 1992; Munns et al., 1993). A few studies, conducted during the reproductive growth of wheat, have focussed on the impact of accumulated ABA on pollen sterility and yield (Dembinska et al., 1992; Ji et al., 2011). The role of leaf ABA on regulating water use by reducing transpiration through stomatal closure and its effect on wheat yield is unknown. Therefore, the overall aim of this thesis was to determine whether ABA-mediated stomatal response to water deficit controls water use to reduce the yield gap under terminal drought conditions.

Chapter 2 reviews the literature and summarises the development of terminal drought and crop response and adaptation when the root system is exposed to drying soil. The focus is mainly on wheat’s ability to regulate water use through regulating stomatal conductance. The review identifies the importance of exploring the role of ABA in stomatal regulation in response to terminal drought conditions, how root distribution affects ABA signalling and subsequent stomatal regulation and how this affects grain yield.

Chapter 3 describes a glasshouse experiment conducted to identify the physiological response of four wheat genotypes with putative tolerance to terminal drought. A paper with information from this chapter was published in 2014 in ‘Field Crops Research’, volume 162 (Appendix 1). The chapter reports differences between the genotypes in leaf ABA accumulation and stomatal closure when half of the root system (split vertically) was exposed to drying soil. Chapter 4 describes how two of the most contrasting genotypes responded when grown in segmented pots that exposed the top part of the root system to drying soil while water was available for deeper roots to extract. The contrasting genotypes differed in their stomatal response to soil water which possibly arose from the different
Chapter 1 – General introduction

signal strengths associated with root distribution in the upper drying soil layer. Chapter 5 describes a transpiration bioassay to identify differential sensitivity of stomata in two wheat genotypes to exogenously applied ABA. ABA feeding could initiate stomatal regulations similar to that in response to drying soil, but only at high concentrations indicating that xylem ABA may not be the sole driver of stomatal regulation. Chapter 6 describes how a root system with better capacity of extracting available water at depth during post-anthesis can minimise grain yield reduction under terminal drought.

Chapter 7, the general discussion, integrates the key findings from the experimental program (Chapters 3, 4, 5 and 6) and explores the implications of the key findings for improving the yield performance of wheat under terminal drought conditions prevalent in Mediterranean-type climatic regions. It also discusses the shortcomings of this research and directions for future research to elucidate ABA-mediated stomatal regulation of water use in wheat under terminal drought.
Chapter 2 – Review of the literature

2 Review of the literature

2.1 Introduction

Wheat (Triticum aestivum L.) is the second most important dietary intake grain after rice (FAO, 2013). Average annual global production is around 650 million metric tonnes, from which 22 million metric tonnes are produced in Australia (FAO, 2013). The annual value of Australia wheat production is about $7 billion and more than 80% of the produce is being exported (ABARES, 2013). In Australia, wheat is mainly grown in a narrow crescent called the ‘grain belt’, stretching from central Queensland through New South Wales, Victoria and southern South Australia and south-western Western Australia (ABS, 2012) (Figure 2.1)

Figure 2.1 Primary wheat growing regions (grain belt) in Australia
2.2 Water deficit: major stress affecting wheat production in Australia

Wheat production in Australia has steadily increased from an average yield of 1 t/ha during the 1970s and 1980s to 1.5 t/ha in the 1990s, reaching a record average yield of 2.11 t/ha in 2001–02 (ABARES, 2013).

In recent years, drought has decreased yields. For instance, average yield during the seasons 2002–03 and 2006–07 was only 0.9 t/ha and in 2007–08 it was 1.1 t/ha (Figure 2.2) (ABARES, 2013). These years recorded severe drought in eastern Australia and Western Australia (ABS, 2012). Year 2002 was declared a dry year for Victoria (BOM, 2002) as was 2006 for Western Australia and New South Wales (BOM, 2007) and 2007 for Western Australia (BOM, 2008). It is clear that water stress is the major factor affecting wheat yield in Australia (Loss and Siddique, 1994).

Figure 2.2 Average wheat yield in Australia from 1971 to 2013. Black bars represent the years in which average yield dropped below 1 t/ha due to severe drought.

Australia is the second driest continent in the world with more than 80% of the land area receiving less than 600 mm of annual rainfall, most falls less than 300 mm (ABS, 2012). The rainfall distribution pattern varies throughout the Australian grain belt. Most rainfall is
received during summer in the northern region while it is fairly evenly distributed throughout the year in the south-east (ABS, 2012). South-western Western Australia and southern South Australia have a Mediterranean-type climate with wet winters and dry summers (Henson et al., 1989a; Siddique et al., 1990b). Hence the pattern of development of water deficit and its impact on yield varies with the growing region and season.

2.2.1 Terminal drought in Western Australia

Western Australia grows wheat in approximately 5 million hectare area, which constitutes 30% of the Australian grain belt (ABARES, 2013). Annual wheat production in Western Australia is valued AUD 1.8 billion (ABS, 2012) and accounts for 38% of the national produce. More than 75% of annual rainfall in WA is received from May to October (Ludwig and Asseng, 2006) and more than 65% is received during winter (Aschmann, 1973). Rainfed wheat cropping is the only option as there is not sufficient water available for irrigation. Annual rainfall in this area has decreased since the 1970s with a reduction of up to 20% and a 60% reduction by 2050 has been predicted (Pittock, 1993). Due to the low rainfall and high temperatures, increasing frequency of low yield years has been predicted in the Western Australian grain belt (Farre and Foster, 2010).

Crop growth is low during winter due to low temperature and radiation (Palta and Watt, 2009). The low transpiration demand due to low temperature, low vapour pressure deficit (VPD) and low variability in winter rainfall reduces the occurrence of water stress during vegetative growth. Low and erratic rainfall, increased temperatures and VPD, and evaporative demand in spring and early summer leads to soil water shortage, which often causes crop water deficit after flowering (Turner and Asseng, 2005). End-of-season drought or terminal drought is the most significant stress affecting wheat yield (Saini and Aspinall, 1981), but the degree of grain yield reduction depends on the time and rate of development of the crop water deficit (Kobata et al., 1992; Palta et al., 1994). A 50% grain yield
reduction occurred in wheat when terminal drought was induced at flowering (Dias de Oliveira et al., 2013). Under extreme terminal drought conditions, wheat yield can fall below 0.5 t/ha (Asseng et al., 2004). Reduced rainfall predicted during autumn may delay sowing until later in the season and could therefore, further increase the risk of exposure to terminal drought (Farre and Foster, 2010). The impact of water stress on wheat yield is determined by how it affects the physiological processes and conditions in plants, which varies between wheat genotypes (Kramer, 1980).

In water-limited environment, grain yield is a function of water use, water use efficiency and harvest index (Passioura, 1983). Hence, terminal drought can be combated to a considerable extent by breeding new varieties with traits to improve water use efficiency (Turner and Asseng, 2005). Water use efficiency (WUE) describes the biomass accumulated per unit of water consumed, and is often used in different levels and units (Turner, 1986; Tambussi et al., 2007). Reduced water uptake will clearly improve WUE, but will result in reduced yield as per Passioura’s equation described above.

2.3 Crop adaptive strategies to combat terminal drought

End-of-season drought or terminal drought occurs when crop enter their reproductive growth stage (Turner and Begg, 1981). Since wheat is a determinate crop (Atwell et al., 1999), adaptation mechanisms such as reductions in leaf area, tiller number and biomass are no longer feasible under terminal drought. Drought escapism, the ability to complete a lifecycle before severe plant water deficit develop (Kramer, 1980), has been used by crop breeders for earliness (Siddique et al., 1989). However, earliness may reduce yield potential in years where rainfall is plentiful (Turner, 1986). Furthermore, under Mediterranean-type climates, drought escapism should be accompanied by low temperature tolerance (Kramer, 1980). Under prevailing unpredictable rainfall conditions, adaptive measures to tolerate drought either by postponing or enduring dehydration (Turner, 1986)
help to sustain physiological activities and minimise yield loss in instances where rainfall is minimal. Osmotic adjustment to tolerate dehydration has no direct influence on grain yield other than modifying water extraction pattern (Morgan and Condon, 1986; Serraj and Sinclair, 2002).

Terminal drought affects grain filling (Fischer and Kohn, 1966; Saini and Aspinall, 1981; Rajala et al., 2009), resulting in shrivelled grains (Mitchell et al., 2013). The carbohydrate requirement for grain filling is partly met by current assimilates and partly by translocation of assimilates stored in vegetative parts. Under terminal drought, the major source of carbon for grain filling is stored assimilates in the tillers (Pheloung and Siddique, 1991; Kobata et al., 1992; Blum, 1998) as photosynthesis will be limited by water stress. The proportion of biomass converted to grain yield is determined mainly by the water used after anthesis (Passioura, 1983). Thus, every extra millimetre of water extracted during the grain filling can result in yield advantage (Manschadi et al., 2006; Kirkegaard, 2007). Therefore, sustaining water uptake during grain filling is critical to improve grain yield under terminal drought. Plant strategies like stomatal closure to limit water loss and/or root properties to slow down soil moisture depletion may protract physiological processes for yield improvement under terminal drought.

2.3.1 Stomatal regulation: an important mechanism to control water use under terminal drought

More than 90% of water uptake in plants is lost through transpiration (Pei et al., 1998) mainly through diminutive pores in the leaf epidermis called stomata. Leaf transpiration is determined by leaf to air vapour pressure deficit and the resistance to the movement of water from leaf to the atmosphere (Farquhar and Sharkey, 1982). Reducing the width of the stomatal opening reduces the ease with which water passes from the plant to the
atmosphere (stomatal conductance) and is considered as a drought adaptive mechanism (Schmidt, 1983).

2.3.1.1 Stomatal regulation is governed by soil water status, not leaf water status

Opening and closing of stomata is controlled by two specialised cells called guard cells that encompass the stomata and is facilitated by changes in turgor inside guard cells (Zelitch, 1967). Guard cells adjust their turgor by the influx or efflux of organic ions like $K^+$, $H^+$ and $Cl^-$ and metabolism of organic acids like malic acid (Raschke, 1975). An increase in guard cell turgor results in widening of stomata (stomata open) and loss of water results in narrowing (stomata close). Hence it was initially believed that under water stress, stomata regulate in response to reduction in leaf water status or turgor (Jones, 1998) that arises due to decreased water supply from the root. Under field conditions, leaf water status declines earlier than root water status (Kramer, 1988). However, genotypes may differ in their ability to regulate stomata in response to their leaf water status. For example, isohydric genotypes initiates stomatal closure to maintain leaf water status while stomatal regulation is triggered by a reduced leaf water potential in anisohydric genotypes (Tardieu et al., 1996; Schultz, 2003; Gallé et al., 2013). Root pressurisation and split – root experiments, in which leaf water status could be maintained, showed that stomatal conductance decreased with soil dryness irrespective of leaf water status (Gollan et al., 1986; Passioura, 1988a). Most research supported soil water status as the prime factor affecting stomatal closure (Aston and Lawlor, 1979; Schulze, 1986; Schulze et al., 1988; Saab and Sharp, 1989; Zhang and Davies, 1990a) and it is now generally accepted that root sense the drying soil and sends signals to the shoot to which stomata responds and regulate the opening to minimise water loss (Gollan et al., 1986; Blum and Johnson, 1993).
2.3.1.2 Root-to-shoot signalling to regulate stomata

Stomatal regulation in response to soil dryness indicates communication between the drying soil and responding leaves. As roots are in direct contact with the drying soil, it has been postulated that roots generate and transmit signals to the leaves such that the stomata respond (Gollan et al., 1986; Passioura, 1988b; Blum and Johnson, 1993). The involvement of root signals in controlling stomata has been confirmed by many studies and a vast pool of data supports a chemical signal, the plant hormone abscisic acid (ABA) (Loveys and Kriedemann, 1974; Zhang et al., 1987; Henson et al., 1989b; Zhang and Davies, 1990a; Munns and Sharp 1993).

ABA has been strongly advocated as the chemical signal involved in this root-to-shoot communication process, but it has not been confirmed as the sole signal involved. For example, Munns and King (1988) showed the presence of a compound different to ABA in the xylem sap of wheat plants that reduces stomatal conductance and increases leaf ABA concentration. In recent years, hormone interactions (Acharya and Assmann, 2009) and interactions between hormones and the environment have also attracted much interest. Thus, the involvement of other hormones and chemicals like cytokinin, auxins, ethylene, jasmonic acid, salicylic acid, H$_2$O$_2$ and ionic substances has been suggested which can act either as positive (presence or increased concentration causes stomatal closure) or negative (absence or decreased concentration reduces stomatal conductance) signals (Schachtman and Goodger, 2008; Acharya and Assmann, 2009; Wilkinson et al., 2012). Esters of ABA, especially glucose esters, can play a significant role as a root signal (Munns and Sharp 1993; Sauter et al., 2002). An increase in xylem pH (Davies and Zhang, 1991; Sobeih et al., 2004) has also been considered as a root signal or an amplifier of root signal which facilitates redistribution of sequestrated leaf ABA to reach guard cells.
Chapter 2 – Review of the literature

A recent study with grafted *Arabidopsis* plants with either ABA-deficient stock or scion point to little importance of ABA as a root signal, but emphasise the importance of leaf ABA in stomatal regulation (Christmann et al., 2007). Supplying water directly to leaves of water stressed plants reverted stomatal closure indicating that hydraulic signals were also involved in stomatal regulation (Comstock, 2002; Christmann et al., 2007). A drop in root water potential, with a net result of decreased soil water potential and water flux, can be considered the signal generator to regulate stomata (Tardieu et al., 1991). No consensus has been reached regarding the root signal that causes stomatal closure when soil dries. Whatever it may be, ABA concentration in wheat leaves increases in response to water stress (Wright, 1969) and modulates stomatal conductance (Mittelheuser and Van Steveninck, 1969).

2.3.1.3 Role of ABA in stomatal regulation

An increased concentration of ABA in leaves associated with reduced stomatal conductance (gs) under water stress has been confirmed by several studies conducted in various species including wheat (Wright, 1969; Loveys and Kriedemann, 1974; Quarrie and Jones, 1977; Quarrie, 1980; Blackman and Davies, 1985; Zhang et al., 1987; Henson et al., 1989b; Davies and Zhang, 1991; Munns and Sharp 1993). Leaf ABA as the main driver of stomatal regulation was questioned when several studies in species like maize demonstrated that xylem ABA increases much earlier than leaf ABA and correlated better with gs than leaf ABA (Blackman and Davies, 1985; Zhang and Davies, 1990a; Tardieu et al., 1992b). This is because leaf ABA consists of ABA sequestrated into the mesophyll chloroplast which has no effect on stomatal regulation (Dodd et al., 1996). However, this is not clearly demonstrated in wheat probably because very few studies have measured xylem sap ABA in wheat under drying soil conditions (Table 2.1) due to the difficulty in obtaining xylem sap (Cramer and Lewis, 1993; Munns et al., 1993). In addition, strong correlation between
leaf ABA and $g_s$ has been demonstrated in wheat (Henson et al., 1989b; Ali et al., 1998), unlike in maize and sunflower (Zhang and Davies, 1990b; Tardieu et al., 1992b).

This does not suggest that xylem ABA has no role in stomatal regulation in wheat. The limited studies that have extracted xylem sap from wheat seedlings by pressuring the whole plant have demonstrated that xylem sap ABA increases with reduction in soil moisture, and turgid wheat leaves reduced $g_s$ when fed the collected sap (Munns and King, 1988; Munns et al., 1993). Wheat leaves fed with exogenous ABA also mimicked the effect of water stress by closing their stomata (Mittelheuser and Van Steveninck, 1969; Quarrie and Jones, 1977), confirming the involvement of xylem ABA in stomatal regulation. Nevertheless, the exogenous ABA concentration required to mimic stomatal response was 100 times that of its endogenous ABA (Munns and King, 1988) indicating that other factors act in conjunction with xylem ABA in stomatal closure, such as the presence of other compounds (Munns et al., 1993) or xylem sap pH (Wilkinson and Davies, 1997; Sobeih et al., 2004).

Alternatively, leaf ABA may also contribute to ABA that reach guard cells in water stressed plants (Cowan et al., 1982; Bahrun et al., 2002), especially in mature plants since stomatal sensitivity to xylem ABA decreases with ageing in wheat (Atkinson et al., 1989). Increased accumulation of leaf ABA in non-pressurised plants compared to pressurised wheat plants under similar moisture stress supports leaf as the major source of ABA at the reproductive stage (Westgate et al., 1996). Flag leaf ABA increases in response to turgor loss and is the source for ABA to the spike (Morgan and King, 1984). Hence at least in wheat plants at the reproductive stage, leaf ABA is significant and a close correlation between leaf ABA and $g_s$ exists (Henson et al., 1989b). Evidence from different species including wheat suggests that stomatal regulation can be considered the net result of an integrative response of both root and leaf ABA (Tardieu and Davies, 1993).
Table 2.1 Examples of previous research conducted in wheat to elucidate the role of ABA under drought

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Stage of plant</th>
<th>Methodology of drought initiation</th>
<th>Tissue sampled for ABA analysis</th>
<th>Exogenous ABA application</th>
<th>Application method</th>
<th>Concentration of exogenous ABA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Seedling</td>
<td>Wilting excised leaf</td>
<td>leaf</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>(Wright, 1969)</td>
</tr>
<tr>
<td>2</td>
<td>Seedling</td>
<td>Withholding water</td>
<td>-</td>
<td>Yes</td>
<td>Injection to leaf sheath</td>
<td>$3.8 \times 10^{-4}$ M</td>
<td>(Quarrie and Jones, 1977)</td>
</tr>
<tr>
<td>3</td>
<td>Vegetative</td>
<td>Withholding water</td>
<td>leaf</td>
<td>Yes</td>
<td>Soil drenching</td>
<td>$10^{-6}$ M</td>
<td>(Du et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>Reproductive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Reproductive</td>
<td>Withholding water</td>
<td>spike</td>
<td>Yes</td>
<td>Injection through leaf sheath</td>
<td>$10^{-4}$ M</td>
<td>(Ji et al., 2011)</td>
</tr>
<tr>
<td>5</td>
<td>Seedling</td>
<td>No drought treatment</td>
<td>Xylem sap</td>
<td>Yes</td>
<td>Added to nutrient medium</td>
<td>$10^{-5}$ M</td>
<td>(Kudoyarova et al., 2011)</td>
</tr>
<tr>
<td>6</td>
<td>Reproductive</td>
<td>Water stress in field</td>
<td>-</td>
<td>Yes</td>
<td>Foliar sprays</td>
<td>$10^{-3}$ M</td>
<td>(Travaglia et al., 2010)</td>
</tr>
<tr>
<td>7</td>
<td>Reproductive</td>
<td>Water stress in field</td>
<td>-</td>
<td>Yes</td>
<td>Foliar sprays</td>
<td>$300$ mg L$^{-1}$</td>
<td>(Travaglia et al., 2007)</td>
</tr>
<tr>
<td>8</td>
<td>Seedling</td>
<td>No drought treatment</td>
<td>Sap and root</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Flag leaf</td>
<td>Withholding water</td>
<td>Flag leaves, floral organs</td>
<td>No</td>
<td></td>
<td></td>
<td>(Vysotskaya et al., 2003)</td>
</tr>
<tr>
<td>10</td>
<td>Stem elongation</td>
<td>No drought treatment</td>
<td>-</td>
<td>Yes</td>
<td>Detached leaf feeding Root medium</td>
<td>$10^{-4}$ M</td>
<td>(Blum and Sinmena, 1995)</td>
</tr>
<tr>
<td>Sl. No.</td>
<td>Stage of plant</td>
<td>Methodology of drought initiation</td>
<td>Tissue sampled for ABA analysis</td>
<td>Exogenous ABA application</td>
<td>Application method</td>
<td>Concentration of exogenous ABA</td>
<td>Reference</td>
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<td>-----------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>11</td>
<td>Seedling</td>
<td>No drought treatment</td>
<td>-</td>
<td>yes</td>
<td>Detached stem feeding</td>
<td>$10^{-3}$M</td>
<td>(Dodd and Davies, 1994)</td>
</tr>
<tr>
<td>12</td>
<td>Seedling</td>
<td>Withholding water</td>
<td>Xylem sap</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>(Munns et al., 1993)</td>
</tr>
<tr>
<td>13</td>
<td>Reproductive</td>
<td>Withholding water</td>
<td>Spikelet</td>
<td>Yes</td>
<td>Through a wick threaded through peduncles</td>
<td>500µL</td>
<td>(Dembinska et al., 1992)</td>
</tr>
<tr>
<td>14</td>
<td>Reproductive</td>
<td>Withholding water</td>
<td>Flag leaves</td>
<td>Yes (to lupin)</td>
<td>Excised leaf feeding</td>
<td>$10^{-4}$ to $10^{-2}$ mol m$^{-3}$</td>
<td>(Henson et al., 1989a)</td>
</tr>
<tr>
<td>15</td>
<td>Seedling</td>
<td>No drought treatment</td>
<td>-</td>
<td>Yes</td>
<td>Injection into mid vein of leaf</td>
<td>$10^{-2}$ and $10^{-3}$ mol m$^{-3}$</td>
<td>(Atkinson et al., 1989)</td>
</tr>
<tr>
<td>16</td>
<td>Reproductive</td>
<td>Withholding water</td>
<td>Leaves</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>(Morgan and King, 1984)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spike</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Reproductive</td>
<td>Withholding water</td>
<td>Leaves</td>
<td>Yes</td>
<td>Immersing leaf in ABA solution</td>
<td>10 and 30 mg L$^{-1}$</td>
<td>(Morgan, 1980)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spikes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Reproductive</td>
<td>Withholding water</td>
<td>Leaves</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td>(Innes et al., 1984)</td>
</tr>
</tbody>
</table>
2.3.1.4 ABA dynamics in plants

ABA is synthesised in root apical cells and also in mesophyll cells in the leaves (Hartung et al., 2002). Plant roots absorb ABA and its conjugates (ABA-glucose ester) from the soil solution (Hartung et al., 2002). Root cells synthesise ABA when their water status is reduced by 50% or more (Hartung et al., 2002). ABA in the root tissues takes both apoplastic and symplastic pathways to reach xylem (Hartung et al., 2002). Xylem ABA acts as an early signal that initiates stomatal regulation (Zhang and Davies, 1990a). As water deficit increases, ABA biosynthesis in leaves is triggered by a reduced leaf water potential or turgor (Westgate et al., 1996). ABA concentration increases in all leaf tissues including guard cells (Harris et al., 1988). Leaf-synthesised ABA is loaded into the phloem and transported to the roots (Slovik et al., 1995; Kudoyarova et al., 2011) either to enter the xylem (Liang et al., 1997) or to be deposited in root tissues (Hartung et al., 2002). During transportation from root to leaves, stem parenchyma cells also contribute to xylem ABA under conditions of high concentration and pH gradient (Sauter and Hartung, 2002). ABA is a weak acid (Hartung and Slovik, 1991), so xylem ABA reaching the leaf lamina gets sequestrated into alkaline compartments of leaf tissues (Cowan et al., 1982; Slovik et al., 1995) depending on the pH gradient between the tissue and xylem (Wilkinson and Davies, 1997). With higher xylem sap pH, ABA sequestration to leaf tissue is reduced or the redistribution of leaf tissue ABA to reach guard cells is favoured (Cowan et al., 1982; Popova et al., 2000). In addition, guard cells can synthesise ABA (Bauer et al., 2013).

ABA also gets degraded to form phaseic acid (PA), which may be further metabolised to dihydrophaseic acid (DPA) (Harrison and Walton, 1975; Creelman and Zeevaart, 1984). Alternatively, ABA conjugates with glucose to form ABA-glucose ester (ABA-GE) which is not active in stomatal regulation (Zeevaart and Creelman, 1988). Esters of ABA are present in the xylem sap of several species (Jeschke et al., 1997; Hansen and Dörfling, 1999; Sauter et al., 2002) and is believed to be involved in root-to-shoot signalling. In
wheat, the high-molecular weight compound with anti-transpiration properties in the xylem sap of water-stressed plants is possibly glucose esters of ABA (Munns and King, 1988; Munns et al., 1993). ABA-GE is capable of releasing free ABA upon hydrolysis by β-glucosidases (Dietz et al., 2000; Lee et al., 2006; Schroeder and Nambara, 2006; Xu et al., 2012). Thus, bulk leaf ABA is the net result of ABA transported through xylem, biosynthesis in leaves, degradation and conjugation (Figure 2.3).

2.3.1.5 Tissue sampling for ABA analysis

A strong correlation exists with ABA and $g_s$, but the relationship varies with species and the tissue sampled (section 2.3.1.3). The main reason for this discrepancy is that ABA concentration in the leaf tissue or xylem sap does not relate to those reaching guard cells, which acts on stomata (Munns and Sharp 1993). The most accurate option is to measure ABA nearest to the site of action, which is the guard cells, but this is possible only in some species like *Commelina* where the epidermis can be stripped easily (Blackman and Davies, 1983) and guard cells can be isolated by killing all other epidermal cells by a low pH treatment (MacRobbie, 1980). Due to this limitation in isolating the epidermis in some species and laborious process involved in collecting sufficient epidermal tissue for analysis, xylem sap is considered the best option to explain stomatal behaviour (Dodd et al., 1996).

Xylem sap sampling is not easy in cereals as it is difficult to collect sufficient volume of xylem sap to quantify ABA (Dodd and Davies, 1996), especially in wheat and barley (Cramer and Lewis, 1993; Munns et al., 1993). Hence, most studies where xylem sap ABA is measured have been conducted on maize due to ease of extraction of a large volume of sap (Dodd and Davies, 1996) (Table 2.2). Besides, much attention is needed to get a representative sample as ABA concentration varies with the volume of the collected sap (Borel and Simonneau, 2002), the method of collection (Quarrie and Lister, 1983), the
point of collection (Netting et al., 2012) and the time of collection (Schurr et al., 1992; Tardieu and Davies, 1992).

2.3.1.5.1 Xylem sap sampling techniques

A commonly used method to collect exuded root xylem sap is from detached root stumps (Zhang and Davies, 1990a; Vysotskaya et al., 2003). Removing the aerial part will cease transpiration, which increases root pressure thus causing exudation. However, little exudate was collected from wheat grown under hydroponics, even without water stress (Cramer and Lewis, 1993). Root exudation did not yield any sap from barley plants also (Martin-Vertedor and Dodd, 2011). Nevertheless, Ali et al. (1998) collected xylem sap from water-stressed wheat plants grown in lysimeters through root exudation. The drawback in exudate collection is that the flux of sap exudation will be much lower due to lack of transpiration pull (Schurr, 1998), which alters the ABA concentration in the sap (Else et al., 1994; Goodger et al., 2005). Information about fluxes and ABA concentration is needed (Schurr, 1998) to account for the changes in stomatal opening. It is ideal to collect the sap when the flow rate is similar to the transpiring rate of an intact plant (Else et al., 1995), but negative pressure in the xylem of transpiring plants make collection extremely difficult (Schurr, 1998).

The pressurisation technique allows sap collection to occur at a similar rate of flux as in intact transpiring plant (Munns et al., 1993), but the ABA concentration may vary due to wounding (Else et al., 1994) and the interruption of the flow of signals and ions from phloem to xylem (Schurr, 1998). Pressurisation can be applied to extract sap from other plant parts like leaves, but the volume of extraction without water contamination from internal compartments is limited (Dodd, 2007). Some studies have collected xylem sap from wheat by pressurising the whole root system in a pressure chamber (Table 2.2). The
lack of pressure chambers (Dodd, 2007) suitable for large flowering stage plants limits its applicability to seedlings.

The application of external forces such as a vacuum is another method of collecting xylem sap. As the xylem fluid is under less axial resistance compared to fluid in surrounding tissues, application of a slight force will separate xylem sap thereby minimising the risk of contamination with other fluids. Applying negative pressure through a vacuum stimulates conditions similar to intact transpiring plants (Freundl et al., 1998). However, few experiments have used this technique to extract xylem sap (Table 2.2).

Controversy exists regarding which xylem sap sampling procedures best represent ABA concentration in the xylem sap of a transpiring plant. As the site of action is on leaves and due to ease of access and abundance (Dodd et al., 1996), leaf tissue is the most sampled in studies on wheat (Table 2.1 and 2.2).
Table 2.2 A summary of literature showing methodology used to collect xylem sap from different species

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Species</th>
<th>Stage of plant</th>
<th>Drought</th>
<th>Pot/field conditions</th>
<th>Sap collection technique</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wheat</td>
<td>Flag leaf stage</td>
<td>Yes</td>
<td>Lysimeter</td>
<td>Root exudation</td>
<td>-</td>
<td>(Ali et al., 1998)</td>
</tr>
<tr>
<td>2</td>
<td>Wheat, barley</td>
<td>Seedling</td>
<td>Yes</td>
<td>Pots</td>
<td>Whole pot in pressure chamber</td>
<td>Difficult from wheat</td>
<td>(Munns et al., 1993)</td>
</tr>
<tr>
<td>3</td>
<td>Wheat</td>
<td>Seedling</td>
<td>Yes</td>
<td>Pots</td>
<td>Whole pot in pressure chamber</td>
<td>-</td>
<td>(Munns and King, 1988)</td>
</tr>
<tr>
<td>4</td>
<td>Wheat, barley</td>
<td>Seedling</td>
<td>Yes</td>
<td>Pots</td>
<td>Whole pot in pressure chamber</td>
<td>-</td>
<td>(Munns, 1992)</td>
</tr>
<tr>
<td>5</td>
<td>Wheat, maize</td>
<td>One month old plant</td>
<td>No</td>
<td>Hydroponics</td>
<td>Wheat: Pressurising shoots, Maize: root exudation</td>
<td>Wheat did not yield any root exudates</td>
<td>(Cramer and Lewis, 1993)</td>
</tr>
<tr>
<td>6</td>
<td>Durum wheat</td>
<td>Seedlings</td>
<td>No (roots severed)</td>
<td>Hydroponics</td>
<td>Root exudation</td>
<td>Cut stump reunited with stem by a tubing</td>
<td>(Vysotskaya et al., 2003)</td>
</tr>
<tr>
<td>7</td>
<td>Barley</td>
<td>7 days after transplanting</td>
<td>Yes</td>
<td>Pot</td>
<td>Pressurising whole plant in pressure chamber</td>
<td>No sap extraction possible under root pressure</td>
<td>(Martin-Vertedor and Dodd, 2011)</td>
</tr>
<tr>
<td>8</td>
<td>Barley</td>
<td>3 weeks old plant</td>
<td>Yes</td>
<td>Pots</td>
<td>Root exudation</td>
<td>Droplets for pH measurement</td>
<td>(Bacon et al., 1998)</td>
</tr>
<tr>
<td>9</td>
<td>Maize</td>
<td>Seedlings</td>
<td>Yes</td>
<td>Pots</td>
<td>Whole pot in pressure chamber</td>
<td>-</td>
<td>(Liang et al., 1997)</td>
</tr>
<tr>
<td>10</td>
<td>Maize</td>
<td>Flowering</td>
<td>No</td>
<td>Pots</td>
<td>Stem bleeding, Root exudation, aspiration</td>
<td>Bleeding sap often unobtainable</td>
<td>(Canny and McCully, 1988)</td>
</tr>
<tr>
<td>Sl. No.</td>
<td>Species</td>
<td>Stage of plant</td>
<td>Drought Pot/field conditions</td>
<td>Sap collection technique</td>
<td>Remarks</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
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<td>----------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------</td>
<td>----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Maize</td>
<td>Seedlings</td>
<td>Yes Pots</td>
<td>Pressurising cut stem</td>
<td></td>
<td>(Wilkinson et al., 2007)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Maize</td>
<td>4 weeks after sowing</td>
<td>Yes Pots</td>
<td>Root exudation</td>
<td>Maize sap fed to wheat leaves</td>
<td>(Zhang and Davies, 1991)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Maize</td>
<td>Silking</td>
<td>Yes Field</td>
<td>Over pressurising leaves (0.5 MPa)</td>
<td></td>
<td>(Tardieu and Davies, 1992)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Maize, Sunflower</td>
<td>5–6 weeks after sowing</td>
<td>Yes Pots</td>
<td>Centrifugation*&lt;br&gt;Pressurising whole root system (sunflower)&lt;br&gt;Root exudate under root pressure (maize)</td>
<td>Sunflower plants did not yield much exudates under root pressure</td>
<td>(Zhang and Davies, 1990b)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Maize</td>
<td>Seedling</td>
<td>Yes Pots</td>
<td>Root exudation</td>
<td></td>
<td>(Zhang and Davies, 1990a)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Maize</td>
<td>Silking</td>
<td>Yes Field</td>
<td>From leaf by over pressurising (0.5 MPa ) after leaf water potential measurement</td>
<td></td>
<td>(Tardieu et al., 1992b)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Maize</td>
<td>28 days after emergence</td>
<td>Yes Lysimeter</td>
<td>Root pressure</td>
<td></td>
<td>(Bahrun et al., 2002)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Sunflower</td>
<td>Seedling</td>
<td>**PRD Pots</td>
<td>Whole pot in pressure chamber</td>
<td></td>
<td>(Dodd et al., 2008a)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Sunflower</td>
<td>Seedling</td>
<td>PRD Pots</td>
<td>Whole pot in pressure chamber</td>
<td></td>
<td>(Dodd et al., 2008b)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Sunflower</td>
<td>Flowering</td>
<td>Yes Field</td>
<td>From leaf by over pressurising (0.3MPa ) after leaf water potential measurement</td>
<td></td>
<td>(Tardieu et al., 1996)</td>
<td></td>
</tr>
<tr>
<td>Sl. No.</td>
<td>Species</td>
<td>Stage of plant</td>
<td>Drought</td>
<td>Pot/field conditions</td>
<td>Sap collection technique</td>
<td>Remarks</td>
<td>Reference</td>
</tr>
<tr>
<td>--------</td>
<td>---------</td>
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<td>---------</td>
<td>----------------------</td>
<td>-------------------------</td>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>21</td>
<td>Grape</td>
<td>Mature</td>
<td>Yes</td>
<td>Field</td>
<td>From leaf by over pressurising (0.1MPa)</td>
<td>-</td>
<td>(Speirs et al., 2013)</td>
</tr>
<tr>
<td>22</td>
<td>Grape</td>
<td>4 months old plant</td>
<td>PRD</td>
<td>Split roots (two pots)</td>
<td>Whole root system removed from pot and inserted into specially designed pressure chamber</td>
<td>-</td>
<td>(Li et al., 2010)</td>
</tr>
<tr>
<td>23</td>
<td>Tomato</td>
<td>5 – 6 weeks</td>
<td>PRD</td>
<td>Split roots (two pots)</td>
<td>From leaf petiole in pressure chamber From petiole stub and root stub when whole plant was pressurised</td>
<td>-</td>
<td>(Netting et al., 2012)</td>
</tr>
<tr>
<td>24</td>
<td>Tomato</td>
<td>6 weeks after germination</td>
<td>PRD</td>
<td>Split roots (two pots)</td>
<td>From leaf by over pressurising (0.4 MPa for 60 – 120 s) after leaf water potential measurement</td>
<td>-</td>
<td>(Dodd, 2007) (Dodd et al., 2006)</td>
</tr>
<tr>
<td>25</td>
<td>Tomato</td>
<td>6 weeks after germination</td>
<td>PRD</td>
<td>Split root</td>
<td>From leaf by over pressurising (0.2 to 0.4 MPa) after leaf water potential measurement</td>
<td>-</td>
<td>(Sobeih et al., 2004)</td>
</tr>
<tr>
<td>26</td>
<td>Cotton</td>
<td>Fruiting</td>
<td>No</td>
<td>Field</td>
<td>From leaf by over pressurising</td>
<td>-</td>
<td>(Hartung et al., 1988)</td>
</tr>
<tr>
<td>27</td>
<td>Nicotiana</td>
<td>Flowering</td>
<td>Yes</td>
<td>Pots</td>
<td>From leaf by over pressurising (0.5MPa) after leaf water potential measurement</td>
<td>-</td>
<td>(Borel and Simonneau, 2002)</td>
</tr>
</tbody>
</table>

*Centrifugation: Sections of midribs of fully elongated leaves were sealed in 10 cm³ syringe barrels and placed above microcentrifuge tubes which were fitted inside a centrifuge container (Centrifuge: Centaur 2, MSE, UK). Sap was collected in the tubes by centrifuging at a speed of 2500 rev. min – 1 for 10 minutes.

**PRD is partial root drying**
2.3.1.6 How does ABA affect stomatal regulation?

Stomata open or close by a change in the shape of the bordering guard cells due to relative changes in turgor between guard cells and surrounding epidermal cells (Meidner and Mansfield, 1968). Stomata open when guard cell turgor is low compared to epidermal cells and close if it is high. Guard cell turgor is maintained by the influx and efflux of certain ions like $K^+$, $Cl^-$, $H^+$ etc, importantly $K^+$. Studies have shown that ABA closes stomata even in turgid leaves (Mittelheuser and Van Steveninck, 1969; Zhang and Davies, 1991); a reasonable assumption that ABA regulates ion fluxes into and out of guard cells. Elevated levels of ABA around guard cells could initiate the loss of $K^+$ leading to stomatal closure (Mansfield and Jones, 1971). However, whether ABA aids stomatal regulation by inhibiting influx or by facilitating efflux is contradictory. Thus, ABA reportedly affects the efflux of anions rather than inhibiting the influx in Commelina species (MacRobbie, 1981) but inhibits influx in Vicia faba (Horton and Moran, 1972). While, exogenous ABA application in Arabidopsis thaliana did not affect guard cell $K^+$ channels (Aurisano et al., 1993). Thus, mechanisms of ABA-mediated stomatal regulation are not yet clear despite the large number of studies; the data generated to unveil mechanisms underlying ABA-mediated stomatal response to water stress disagree, revealing the complexity of the phenomenon (see below and Figure 2.3).

1. ABA is not the sole chemical involved in stomatal regulation; several other phytohormones and/or compounds are involved and hence stomatal response is the net result of the interaction between several hormones and other environmental factors

2. A multitude of factors determine the accumulation of ABA and stomatal response to a given concentration of ABA: (i) genetic factors (Quarrie, 1980), (ii) water status of plants such as leaf water potential (Quarrie, 1980) or turgor (Morgan and King, 1984), (iii) soil water status, (iv) pH (Slovik et al., 1995; Hartung et al., 2002), (v) soil compaction
(Tardieu et al., 1992b), (vi) environmental factors such as temperature (Wright, 1969; Dodd and Davies, 1994), light or time of day, (vii) changes in water flux through the xylem (Slovik et al., 1995) and (viii) previous exposure to ABA flux (Atkinson et al., 1989).

3. The ABA concentration reaching the guard cells at any given time can vary due to sequestration, remobilisation and degradation and/or conjugation. All the mechanisms are not yet fully understood.

4. ABA is synthesised in several tissues including roots, leaves and guard cells (Bauer et al.). In addition ABA is taken up by roots from the soil (Hartung et al., 2002).

5. ABA is mobile within the plant travelling up and down the plant; from root to leaves through xylem and from leaves to roots through phloem. ABA from leaves is also exported to spikes. Regulation of this movement and its physiological implications is not yet clear (Seo and Koshiba, 2011).

6. The action of ABA is not limited to stomatal regulation, but is involved in many physiological functions from seed germination, growth (Milborrow, 1967), tiller production (Quarrie and Jones, 1977), root hydraulic conductivity (Davies et al., 1982), cell wall rigidity (Davies et al., 1982), pollen sterility (see section 2.4.2), and in determining yield (Travaglia et al., 2007).

The involvement of ABA in stomatal regulation is equivocal, but the above-listed intricacies have limited the understanding of complex mechanism of ABA-mediated stomatal regulation under water stress. Moreover, stomata is controlled by several feedback loops (Raschke, 1975) involving external and internal factors such as light, VPD, intercellular CO$_2$ concentration, leaf turgor and soil water status.
Figure 2.3 Schematic representation of kinetics of ABA through the plant
2.3.2 Root traits to favour terminal drought adaptation in wheat

2.3.2.1 Root distribution: a potential trait to maintain water uptake under drought

Root characteristics, especially distribution patterns and architecture, have a significant role in crop productivity under water deficit conditions (Lynch, 1995; Manschadi et al., 2006). However, research in this area has been limited because of the difficulty in studying root form and function in-situ.

In wheat, root distribution and architecture varies with genotypes (O'Toole and Bland, 1987; Siddique et al., 1990a; Manschadi et al., 2006; Palta et al., 2011) and with soil environment (Bingham and Bengough, 2003; Kirkegaard, 2007). Genotypic variations in root characteristics significantly impact water uptake and yield under water-limited environments (Manschadi et al., 2006). Root traits such as root distribution with depth (Oyanagi, 1994), root length density, xylem vessel diameter (Richards and Passioura, 1989), root-to-shoot ratio (Siddique et al., 1990a) and root vigour (Palta and Watt, 2009; Palta et al., 2011) are often considered favourable in drought-prone environments.

A deeper root system is generally considered important (Oyanagi, 1994; Manschadi et al., 2006; Wasson et al., 2012), as this trait appears beneficial in capturing moisture from deeper soil layers, especially during grain filling (Passioura, 1983). This is particularly true in areas where crop growth depends mainly on stored soil moisture such as the north-eastern grain belt of Australia (Manschadi et al., 2006). In the Mediterranean-type climate of the southern and western grain belt of Australia, where crops rely solely on seasonal rainfall events, a vigorous root system is advocated for increased growth and yield of wheat (Palta et al., 2011).

Crop growth is slow during winter (Palta and Watt, 2009) when more than 65% of annual rainfall is received in the Mediterranean region of Australia (Loss and Siddique, 1994).
Hence water received during winter far exceeds crop demand and drains down the soil profile (Palta and Watt, 2009). Root vigour, early root development and branching, favour effective capture of soil moisture and nutrients. By spring and early summer, the soil starts drying near the surface where most of the roots are concentrated (Turner and Asseng, 2005). Under terminal drought conditions, vigorous roots are less advantageous (Palta et al., 2011) and root growth in the deeper soil layers is considered advantageous (Loss and Siddique, 1994). Therefore under terminal drought conditions, genotypes capable of sustaining root growth post-anthesis to extract additional water from deeper soil profile would be beneficial (Vadez, 2014).

Wheat roots reach maximum growth by anthesis (Siddique et al., 1990a; Gregory et al., 1992) and further growth is generally not noticed. However, post-anthesis root growth is observed in some wheat genotypes such as SeriM82 (Manschadi et al., 2006) and when the primary carbon sink (spikes) are removed from the plant (Richards et al., 2007). This shows that wheat roots can grow beyond anthesis stage in certain circumstances. However, continued root growth will result in unbalanced assimilate partitioning from the shoot to root limiting grain yield (Passioura, 1983; Song et al., 2009). In crops like wheat, which are grown primarily for grains, assimilate diversion to such adaptation is not conducive for improved grain yield. Furthermore, roots below 70 cm in the soil profile take up limited amounts of water (Ali et al., 1998). A deeper root system, therefore does not implicate efficient water extraction from deeper soil profile. Wheat roots do not absorb water uniformly along their root length (Passioura, 1983; Bramley et al., 2009) which may be due to poor root-soil contact (White and Kirkegaard, 2010) or impermeability of root (Passioura, 1983).
2.3.2.2 Root hydraulic resistance: a trait that limits water flux through roots

Water flow through plants is governed by the driving forces and resistance imposed by the conduit (Boyer, 1985). Considerable resistance to water flow through the plant is provided by roots (Newman, 1976). Therefore resistance to water flow (low conductance) within the root prevents absorption and the supply of water to the shoot even though root growth is sufficient to reach available water within the soil.

Water absorbed by roots flows across the root radius to reach xylem (radial pathway) and then follows a longitudinal pathway to the shoot through the xylem (axial pathway). Hence, root hydraulic resistance is a combination of resistances offered by both radial and axial pathways, with radial flow being the greatest constraint (Steudle and Peterson, 1998; Bramley et al., 2009). Root structure and anatomy contributes to the hydraulic properties of roots (Bramley et al., 2009). For instance, small xylem vessels impart larger resistance to water flow through the xylem (Richards and Passioura, 1989). Likewise, the predominant radial pathway adopted affects hydraulic conductance. For example, apoplastic flow is driven by the hydrostatic gradient and involves minimal resistance compared with the symplastic pathway (Steudle and Peterson, 1998). In wheat, significant radial water flow occurs symplastically (Bramley et al., 2009), which is facilitated by the membrane-bound protein, aquaporin. Aquaporin activity can potentially be enhanced by interactions with ABA (Hose et al., 2000). A higher concentration of ABA was observed in wheat roots in association with increased root hydraulic conductance following excision of four out of five seminal roots (Vysotskaya et al., 2003; Vysotskaya et al., 2004). This hike in root ABA and subsequent enhancement of root hydraulic conductivity to meet increased transpiration demand is due to redistribution of ABA from leaf to root (Kudoyarova et al., 2011). Thus leaf ABA is involved in regulating root hydraulic conductivity, in addition to its role in regulating stomata.
2.3.2.3 Root distribution and water use by stomatal regulation

The prime water conservation technique under water stress—stomatal closure — is in response to the signals generated and transmitted from the roots (see section 2.3.1.2). Hence root characteristics might play an important role in this signal generation process. Wheat plants regulated stomata in response to drying signals from the roots in the top drying layer of the soil profile even though leaf water status was maintained by unlimited water supply from deeper soil layers (Blum and Johnson, 1993). These findings were substantiated with increased ABA concentration in barley leaves when more seminal roots were distributed in the dry half of the pots (Martin-Vertedor and Dodd, 2011). This proves that the root distribution plays an important role in signal generation and subsequent stomatal regulation. Therefore, under terminal drought conditions in the Mediterranean-type regions of Australia, a greater root distribution in drying upper soil layers may accumulate ABA in leaves and regulate stomata to conserve water for grain filling. This may help the plant as an early signalling mechanism to regulate stomata and conserve water well before a large part of the root zone has been depleted of water.

2.4 The effect of stomatal regulation on yield

The main objective of any wheat breeding program is yield improvement. Unfortunately, many favourable plant responses to moisture stress have negative effects on grain yield (Schmidt, 1983; Turner, 1986). Blum and Johnson (1993) reported a negative impact on yield of wheat cultivars with reduced stomatal conductance in response to top dry soil. This may be due to reduced photosynthesis due to stomatal closure (see section 2.4.1) or the adverse effect of ABA on pollen sterility as reported by several scientists (see section 2.4.2).
2.4.1 Stomatal conductance and photosynthesis

Stomata serve as a portal through which water exits the leaf and CO₂ diffuses into leaf tissue for photosynthesis. Therefore, stomatal regulation to limit water use during water deficit is at the expense of CO₂ diffusion into leaf tissue which subsequently reduces photosynthesis (Chaves et al., 2009). In addition to the reduced CO₂ influx, soil water deficits reduces mesophyll conductance of CO₂ limiting photosynthesis (Flexas et al., 2004). As the relative water content decreases due to soil water deficit, decreased ATP synthesis and consequent RuBP synthesis causes metabolic limitation of photosynthesis (Lawlor, 2002; Lawlor and Cornic, 2002). Interestingly, the rate of reduction of CO₂ assimilation is comparatively less than the reduction in transpiration (Holaday et al., 1992). In well-adapted plants, the stomatal role in controlling photosynthesis is not more than 20% of the total photosynthetic inhibition (Jones, 1998).

2.4.2 Impact of ABA on yield

Reduction in grain set due to drought has been associated with increased ABA concentration in leaves and spikes (Morgan and King, 1984). Yield reduction in response to exogenous ABA application supports the negative impact of ABA on grain set and yield (Morgan, 1980). A reduction in the number of grains per ear in genotypes selected for high leaf ABA levels even under well-watered conditions suggests that leaf ABA negatively influences wheat pollination (Innes et al., 1984). Therefore yield reduction is observed when the drought coincides with pollen mother cell meiosis and not during any later developmental stage (Saini and Aspinall, 1981). Furthermore, ABA-associated pollen sterility is more pronounced in drought-sensitive wheat genotypes compared to drought tolerant ones (Ji et al., 2011). In a split-root study, where the dry half of the root system contributed to increased leaf ABA while the other half supplied water to maintain leaf
water potential, no yield reduction was noticed (Dembinska et al., 1992). This suggests that endogenous ABA is not the sole factor affecting grain set under drought.

Conversely, ABA is reported to have a positive influence on grain yield by affecting the redistribution of carbohydrates from the shoot into wheat grain (Travaglia et al., 2007). Increased grain yield by foliar application of ABA in a wheat field was confirmed by Travaglia et al. (2010). This disagrees to the findings of King and Patrick (1982) where no such involvement of ABA in assimilate transport to grain was observed. Du et al. (2013) also observed no yield benefit by drenching soil with exogenous ABA. Lack of consensus among researchers with regard to positive, negative or neutral influences of ABA on grain yield suggests that the timing of water stress is of utmost importance. Pre-anthesis (during spike development and pollen meiosis) water stress reduced grain number while post-anthesis water stress reduced the grain size (Dolferus et al., 2011). This is because high ABA level at early reproductive stage affects the grain set and reduces grain number while the post-anthesis water stress promotes grain filling by redistributing reserved carbohydrates to the grain (Liu et al., 2005). Based on the above studies, it appears that ABA negatively affects grain set, but has a positive effect on grain filling by facilitating assimilate partitioning to grains.

### 2.5 Summary and research opportunities

Terminal drought is the major abiotic stress affecting wheat grain yield in Mediterranean-type environment in south-western Australia. In this environment, every extra millimetre of soil water extracted during grain filling benefits grain yield and water use efficiency. Stomatal regulation is an important mechanism that controls water use. The role of ABA in regulating $g_s$ under water deficit has been explored extensively in crops such as maize, tomato and sunflower, with few studies in wheat. Most studies in wheat have been conducted during the vegetative growth focussing on the effect of ABA on $g_s$ as an
adaptive mechanism. Not many studies have focussed on the role of ABA in controlling water use during grain filling through regulation of stomatal aperture in relation to grain yield when exposed to terminal drought. ABA reportedly has negative and positive impacts on grain set, depending on the stage during which the plant was exposed to drought. Studies focussing on terminal drought should unravel the mechanisms of ABA-mediated stomatal regulation and its impact on grain yield in wheat.

ABA accumulation in the leaves is initiated through a root-to-shoot signalling process. The signal involved in this process is not clear, but is controlled by the roots in contact with the drying soil. Root being the source of drying signal, root density in the drying soil layer may have an impact on signal strength and subsequent stomatal closure. This has been identified in crops such as barley, but has not studied in wheat. Understanding the link between root distribution and stomatal closure is important to identify traits for efficient management of available resources under terminal drought conditions. The major research gap identified in this review of literature is the lack of understanding on the impact of ABA accumulation and stomatal regulation on wheat water use and grain yield under terminal drought conditions (Figure 2.4).
Chapter 2 – Review of the literature

Figure 2.4 Schematic representation of the research gap identified by this review of literature. Green arrows represent scope of this study in relation to the previous research works in this area (red, yellow and blue arrow).

2.6 Scope of the research

This research focuses on the terminal drought and investigates the stomatal mechanisms in wheat genotypes to adapt without affecting its yield potential. Furthermore, this project investigates root distribution patterns, the least explored, but potentially the most important trait in relation to root-to-shoot signalling associated with soil dryness. The overall aim of this research is to explore the impact of ABA production and regulation of stomatal aperture in regulating water use during grain filling when the wheat plants are exposed to terminal drought. The main hypotheses addressed in this research are as follows:
1. differences in stomatal response to soil water deficit corresponds to increased accumulation of ABA in the leaves

2. more root density distributed in the drying upper soil layer leads to early stomatal closure in response to soil water deficit

3. xylem ABA is the major driver to initiate stomatal closure

4. stomatal sensitivity to xylem ABA concentration differs between genotypes

5. stomatal sensitivity to xylem ABA concentration differs with xylem sap pH and water status of the plant.

6. wheat genotypes differ in their root capability to extract water from the bottom of the soil profile

7. early stomatal closure conserves water for use during grain filling period which reduces yield gap.
3 Contrasting stomatal regulation and leaf ABA concentrations in wheat genotypes when split root systems were exposed to terminal drought

3.1 Introduction

Wheat is one of the world’s staple food crops and is Australia’s most important grain crop (ABS, 2012). Terminal drought which occurs during post-anthesis (Kobata et al., 1992; Loss and Siddique, 1994) significantly reduces wheat grain yield (Saini and Aspinall, 1981; Simane et al., 1993). Due to decreasing rainfall and rising temperatures (Turner and Asseng, 2005), the frequency of exposure to terminal drought is predicted to increase for dryland wheat growing regions (Farre and Foster, 2010). Terminal drought induces grain abortion (Rajala et al., 2009) and affects grain filling (Saini and Aspinall, 1981; Rajala et al., 2009), resulting in shrivelled grains (Mitchell et al., 2013) and thus, reducing both grain yield (Dias de Oliveira et al., 2013) and quality (Gooding and Davies, 1997). Water used during post-anthesis plays an important role in determining wheat yield (Passioura, 1983; Siddique et al., 1990b; Manschadi et al., 2006). Hence, maintaining of water uptake during grain filling is critical (Richards and Passioura, 1989) under terminal drought, which can be achieved by minimising water loss through regulation of stomatal conductance.

Stomatal regulation in response to soil dryness is affected by the root’s ability to sense the drying of surrounding soils and communicate this information to the shoot (Gollan et al., 1986; Passioura, 1988b). Abscisic acid (ABA) is strongly advocated as the major signal involved in this root to shoot communication process (Zhang and Davies, 1987; Dodd, 2005), despite evidence that other chemicals are also involved (Munns and King, 1988; Yao et al., 2013) and/or signals (Davies and Zhang, 1991).

ABA is considered mainly as a root-derived hormone regulating stomatal closure in response to water stress (Slovik et al., 1995; Hartung et al., 2002). However, a lack of
correlation between xylem ABA and stomatal conductance (Atkinson et al., 1989; Ali et al., 1998), but a better correlation with leaf ABA (Henson et al., 1989b) indicates that stomatal regulation in response to soil dryness is related to the accumulation of ABA in leaf tissues (Mahdid et al., 2011), at least in wheat.

Wheat genotypes differ in their ability to produce ABA (Quarrie, 1980; Quarrie and Henson, 1981) and in regulating stomata (Blum and Johnson, 1993) when exposed to drying soil and hence, may differ in their ability to save water. As the soil starts drying, the top part of the soil profile begins to dry while soil moisture is still available at depth. Thus, the part of the root system in the top soil profile will experience low soil water potential (Passioura, 1988a) while the deeper part of the root system has access to soil moisture (Zhang and Davies, 1990a). Therefore, although water may still be available at depth, roots in the drier part of the soil profile may produce a signal that induces ABA production and the subsequent increase in ABA concentration in leaves may cause partial stomatal closure and water conservation. This behaviour has been observed in some horticultural crops and where partial root-zone or deficit irrigation is used (Stoll et al., 2000; Sobeih et al., 2004), but it is not known if this occurs in wheat. Wheat genotypes may differ in root system characteristics that detect and signal drying soil conditions, particularly since wheat root systems are comprised of two types of roots and there is genotypic variation in root system architecture (Manschadi et al., 2008). This experiment was conducted as part of a larger study examining mechanisms of water conservation involving stomatal regulation and ABA production by roots in response to soil drying in wheat. To mimic partial root-zone drying, a split-root system was used to expose half of the root system to terminal drought–drying soil from anthesis. The dry half of the root system simulates roots exposed to low soil water potential (shallow roots) while the wet half simulates roots exposed to high soil water potential (deeper roots under field conditions). This approach was more feasible for an initial screening study to identify contrasting responses in stomatal regulation and ABA
Chapter 3 – Stomatal regulation and leaf ABA

production to terminal drought rather than the large, deep pots that would be required to simulate terminal drought in the vertical soil profile. Four genotypes were selected for comparison based on their adaptation to contrasting environments in soil moisture (two commercial cultivars) or with putative drought tolerance based on yield performance across a range of dryland environments (two advanced breeding lines). We expected genotypic differences in stomatal closure corresponding to difference in ABA concentration in leaves in response to soil drying around half of the root system.

3.2 Materials and methods

3.2.1 Plant material

Four wheat (*Triticum aestivum* L.) genotypes, including two commercial cultivars (Drysdale and Wyalkatchem) and two advanced breeding lines (IGW-3119 and IGW-3262) putatively adapted to drought were grown in specially-designed split pots. The cultivar Wyalkatchem, commercially released in 2001, is one of the most common cultivars currently grown in Western Australia and is adapted to low rainfall regions (Penny, 2002). The wheat-belt in south-west Western Australia has predominantly intermittent rainfall and sandy-loam soils. The cultivar Drysdale, commercially released in 2002, has high leaf level transpiration efficiency based on its low discrimination against the carbon isotope $^{13}$C compared with $^{12}$C (Condon et al., 2004). This cultivar was designed for environments with predominantly stored soil moisture. The advanced breeding lines IGW-3119 and IGW-3262, provided by Dr. Dan Mullen from InterGrain Pty Ltd. are putatively adaptable to dryland environments (D. Mullen, InterGrain, pers. comm.).

3.2.2 Split pot and growing conditions

Split pots were designed to split the root system in two halves and maintain one half in moist soil while allowing the other half to dry. The split pots were constructed from 30 cm long and 15 cm diameter polyvinyl chloride columns, cut vertically into two equal halves.
A vertical plastic partition wall was placed between the two halves before rejoining using waterproof tape (Figure 3.1). The width of the vertical wall was 2 mm greater than the outer diameter of the pot and its bottom was so aligned that it was 1 mm greater than the bottom of the pot to keep the two compartments hydraulically isolated. The whole assembly was held tight using cable ties.

**Figure 3.1** Diagram of the specially-designed split pots used to expose different halves of wheat root systems to water deficit at anthesis (a). Partition wall fixed on one half of the pot (b). A flexible tube held the germinated seed in place (inset) so that roots could be divided equally into each compartment of the pot (c).

Both halves of each pot were filled with a uniform soil mixture to a depth of 26 cm. The soil was a reddish-brown sandy clay loam obtained from a field site at Bindi Bindi in the Western Australian grainbelt, mixed with 40% coarse river sand to improve drainage. The soil–sand mixture (approximately 9 kg per pot) had a pH of 7.8, electrical conductivity of 0.0126 S m$^{-1}$ and water holding capacity of 0.25 L kg$^{-1}$. Nutrients were supplied through slow release fertiliser pellets (Osmocote®, N:P:K 13:1.5:4.9) mixed into the top 0.1 m soil before planting at the rate of 10g per pot and weekly application of water soluble fertiliser (Haifa Poly-feed, N:P:K 19:8.4:15.8 with magnesium and micronutrients) was applied until
anthesis. Uniform sized seeds were germinated in Petri dishes on moist filter paper at room temperature for three days. Germinated seeds were transferred individually to a 2.0-cm long flexible tube, which was fixed in a groove on the top edge of the partition between the pot compartments (Figure 3.1). The seminal roots were carefully diverted into the two compartments. In order to avoid dehydration of roots due to exposure, the base of the plant was covered with aluminium foil until the plant established. Every 2–3 days, emerging new roots were carefully diverted to both compartments, to ensure that root growth was equally distributed in both compartments. The four genotypes were grown in an evaporative-cooled glasshouse at The University of Western Australia, Perth, WA (31° 93´S, 115° 83´E) from June to October 2011. The mean minimum and maximum temperatures in the glasshouse during the experiment were 12 ± 2 and 26 ± 2 °C, respectively and mean relative humidity was 52 ± 6 %. The four genotypes were grown on five benches in a completely randomised block design with five replicates. Pots were rotated weekly to minimise spatial variability. The pots were watered on alternate days close to pot water holding capacity until anthesis—Z65, Zadoks’ growth scale for cereals (Zadoks et al., 1974)—when the wet and dry treatments were applied. Anthesis for each genotype was determined separately when anthers had emerged from the main stem of 50% of plants.

3.2.3 Treatments

At anthesis, pots from each genotype were divided into three groups of 10 pots. In the first group, both compartments of each pot were well-watered (WW). In the second group, water was withheld from one compartment in each pot (WD), and in the third group, water was withheld from both compartments of each pot (DD). Wet compartments were watered by hand every day to maintain them close to pot soil water holding capacity. To prevent loss of water from the soil through evaporation, the soil surface in each pot compartment was covered with a 3 cm layer of white plastic beads.
3.2.4 Measurements

Shoot and root biomass was measured in all four genotypes three times during the experiment: (1) at anthesis, before terminal drought was induced, (2) when the plants in the DD treatment showed permanent wilting symptoms, which was at 190 °Cd (with a base temperature of 0 °C) for the IGW lines and 170 °Cd for Drysdale and Wyalkatchem (Figure 3.2), and (3) at the final harvest. Before each sampling, the number of tillers per plant was recorded; the shoots were then cut from the roots at the crown. Leaf area was measured at anthesis and at the second harvest. Measurements were made using a portable leaf area meter (LI-3000, LI-COR Biosciences, Nebraska, USA). Shoot and leaves were separately dried in an oven at 60 °C for 48 hours and then weighed. Immediately after harvesting the shoots, each split pot was opened and the roots in each half were recovered from the soil by repeated gentle washing and sieving on a 1.4-mm sieve to produce a clean sample, as described by Liao et al. (2006) and Palta et al. (2007). Roots were then dried in an oven at 60 °C for 72 hours and weighed. At the second harvest, root length as well as root dry weight was measured after the roots were recovered from the soil in each half. Roots were stained for 30 min with 0.1% (w/v) methylene blue, placed in about 3 mm of water in a glass tray (0.2 x 0.3 m), and untangled with a plastic spatula to minimise overlapping. The glass tray was placed on a flatbed scanner with transparency unit (Epson STD4800). Scanning was done at 400 dpi resolution and the images were analysed for total root length using WinRHIZO Pro 2009b (Regent Instruments Inc., Canada). Root material was then dried and weighed. Specific root length (SRL) was calculated as root length per unit biomass of root.
Chapter 3 – Stomatal regulation and leaf ABA

Figure 3.2 Typical examples of wheat plants at second harvest when watering was withheld from both halves of the split root system (DD), one half of the split root system (WD) and when both halves of the split root system were well-watered (WW).

At final harvest, the number of spikes per plant and spikelets per spike were counted. Spikes were separated from shoots, oven dried at 40 °C for 6 to 7 days and threshed by hand. The number and weight of grains per plant were also recorded. Harvest index (HI) was calculated as the ratio of grain yield to shoot biomass.

Leaf water potential ($\Psi_{\text{leaf}}$), stomatal conductance ($g_s$), transpiration and leaf net photosynthesis rate were measured on the flag leaf of the main stem of five replicate plants just before the second harvest, which was between 81 and 84 DAS (permanent wilting in DD treatment). Measurements were made between 10.30 am and 1.30 pm, on days with
clear sky. Rates of leaf net photosynthesis, \( g_s \) and transpiration were measured with a LI-COR gas-exchange system (LI-6400, LI-COR Biosciences, Nebraska, USA) with LED light source on the leaf chamber. In the LI-COR cuvette, \( CO_2 \) concentration was set to 380 \( \mu \text{mol mol}^{-1} \) and LED light intensity 900 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), which is the average saturation intensity for photosynthesis in wheat (Austin, 1990). Immediately after these measurements were made, \( \Psi_{\text{leaf}} \) was measured using a Scholander pressure chamber (model 1000, PMS Instrument Co., Oregon, USA). The flag leaf was loosely covered with a plastic sheath before excision and during the measurement to avoid evaporation (Turner, 1988).

### 3.2.5 ABA sampling and analysis

After gas exchange and \( \Psi_{\text{leaf}} \) measurements, the same flag leaf was snap frozen in liquid nitrogen and stored at \(-80^\circ \text{C}\). ABA analysis was done on two genotypes contrasting in stomatal conductance in response to soil drying. Analysis was carried out as per the protocol described by Speirs et al. (2013). Briefly, 50–100 mg of ground frozen tissue was extracted overnight at 4 \( ^\circ \text{C} \) in 500 \( \mu \text{L} \) of 20% aqueous methanol. After centrifugation, a deuterated internal standard (400 \( \mu \text{L} \), containing D3–7',7',7'-PA and -dihydrophaseic acid, D5–4,5,8',8',8'-ABA-GE and D6–3',5',5',7',7',7'-ABA, all at a concentration of 10 ng \( \text{mL}^{-1} \)) was added to the supernatants. Phenomenex SPE columns (60 mg \( \text{mL}^{-1} \): 8B-S100-UAK) were equilibrated with 1 \( \text{mL} \) methanol and 1 \( \text{mL} \) nanopure water, as per the manufacturer’s directions. The samples were loaded onto the columns, washed with 20% aqueous methanol (1 \( \text{mL} \)), and eluted with 90% aqueous methanol (1 \( \text{mL} \)). An aliquot (50 \( \mu \text{L} \)) of the eluate was dried in a vacuum centrifuge in preparation for analysis by liquid chromatography/mass spectrometry (LC-MS/MS). The dried leaf extracts were dissolved in 50 \( \mu \text{L} \) aqueous acetonitrile (10% with 0.05% acetic acid) and 20 \( \mu \text{L} \) was analysed by LC-MS/MS (Agilent 6410). Separations were carried out on a Phenomenex C18(2) 75 mm \( \times \) 4.5 mm \( \times \) 5 \( \mu \text{m} \) column at 40 \( ^\circ \text{C} \). Solvents were nanopure water and acetonitrile, both with
0.05% acetic acid. Samples were eluted with a linear 15 min gradient starting at 10% acetonitrile and ending at 90% acetonitrile. Compounds were identified by retention times and multiple reaction monitoring.

3.2.6 Statistical analysis

Two-way ANOVA was conducted using the statistical software R 2.14.0 (R Development Core Team, 2011). To meet the assumptions of ANOVA, normal distribution and equal variance, data were subjected to square root transformation before analysis where necessary. To check if withholding water from one half of the pot affected the distribution of roots between wet and dry compartments, a two-way ANOVA was conducted with genotype and drought treatments (wet and dry) of each compartment as dependent variables. Multiple comparisons were done using the Least Significant Difference (LSD) test using the R package agricolae (Mendiburu, 2010). Wherever an interaction effect was significant, results were interpreted based on the LSD value for interaction and main effects were not taken into account.

3.3 Results

3.3.1 Phenology

All genotypes except the line IGW-3262 reached anthesis at 74 to 75 DAS. IGW-3262 reached anthesis at 71 DAS. When watering was continued in both halves of the split root system until maturity (WW), IGW-3262 was the first to attain physiological maturity (when 90% of the flag leaf turned yellow and glumes lost green colour in 50% of the plants for each genotype) and Wyalkatchem the last (Table 3.1). When water was withheld from one half of the split root system (WD), all genotypes except IGW-3262 reached maturity 9 to 12 days earlier than those plants in which both halves of the root system were well-watered (WW). IGW-3262 plants were similar under both WD and WW treatments. When water
was withheld from both halves of the split root system (DD), genotypes attained physiological maturity 16 to 24 days earlier than under the WW treatment (Table 3.1).

Table 3.1 Number of days to anthesis and physiological maturity in four wheat genotypes grown in pots with the root system split between two compartments, where both halves were well-watered (WW) or watering was withheld from one half (WD) or both halves of the split root system (DD) starting at anthesis. Values for days to anthesis are means (n = 35; P < 0.0001; LSD at 95% level of significance = 1).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Anthesis (DAS)</th>
<th>Physiological maturity (DAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>WD</td>
</tr>
<tr>
<td>IGW-3119</td>
<td>75</td>
<td>139</td>
</tr>
<tr>
<td>Drysdale</td>
<td>75</td>
<td>137</td>
</tr>
<tr>
<td>Wyalkatchem</td>
<td>74</td>
<td>140</td>
</tr>
<tr>
<td>IGW-3262</td>
<td>71</td>
<td>132</td>
</tr>
</tbody>
</table>

3.3.2 Leaf water potential (Ψ_leaf), gas exchange and leaf tissue ABA

There was a significant interaction of genotype x treatment for Ψ_leaf (P = 0.002). Ψ_leaf did not differ between genotypes under well-watered conditions (WW) (mean –1.3 ± 0.05 MPa; Table 3.2). When watering was withheld from one half of the root system (WD) from anthesis, Ψ_leaf decreased to –1.8 MPa in Drysdale and Wyalkatchem, but did not decrease in IGW-3262 and remained around –1.3 MPa, similar to WW plants. Withholding water from both halves of the root system (DD) reduced Ψ_leaf in Drysdale and IGW-3262 to –2.8 MPa, while in IGW-3119 and Wyalkatchem, Ψ_leaf decreased to –3.3 and –3.8 MPa, respectively.

Main effects of genotype and treatment were significant for stomatal conductance (P = 0.02 and P < 0.001, respectively), but the interaction was not significant (P = 0.7). Stomatal conductance (g_s) in all genotypes was similar under well-watered conditions with a mean value of 377 mmol m⁻² s⁻¹ (Table 3.2). Withholding water from one (WD) or both (DD) halves of the root system reduced g_s in all genotypes significantly. Under the WD
treatment, $g_s$ in Drysdale and Wyalkatchem decreased by an average of 35% compared with 27% in IGW-3119 and 20% in IGW-3262. Under the DD treatment, $g_s$ declined by 70% in IGW-3262 and by 90% in Wyalkatchem, Drysdale and IGW-3119.

Transpiration rate was similar among genotypes within each treatment (WW mean = 2.665 ± 0.3 mmol m$^{-2}$ s$^{-1}$; $P = 0.21$; Table 3.2). Withholding water significantly reduced transpiration rate in all genotypes ($P < 0.0001$). It decreased to mean = 1.75 ± 0.3 mmol m$^{-2}$ s$^{-1}$ in WD and mean = 0.21 ± 0.05 mmol m$^{-2}$ s$^{-1}$ in DD treatments.

There was significant interaction effect of genotype x treatment for leaf net photosynthetic rate ($P = 0.01$). Net photosynthetic rate was 10.4 μmol m$^{-2}$ s$^{-1}$ in all genotypes under the WW treatment (Table 3.2). Under the WD treatment, leaf net photosynthetic rate decreased by 70% in Drysdale, 40% in both Wyalkatchem and IGW-3119, but only by 15% in IGW-3262 ($P < 0.01$). Leaf net photosynthesis in all genotypes was zero when watering was withheld from both halves of the root system (DD).

The genotypes Drysdale and IGW-3262 had contrasting responses to water stress, but were expected to have comparable soil moisture use pattern due to their similar leaf area and root biomass. Hence ABA analysis was done only in Drysdale and IGW-3262. There was a significant interaction of genotype x treatment for leaf ABA ($P = 0.001$). Leaf ABA concentration increased two to three-fold when watering was withheld from one half (WD) or both halves (DD) of the root system in Drysdale (Figure 3.3). In IGW-3262, there was no increase in leaf ABA under the WD treatment, but it increased seven-fold under the DD treatment. Leaf ABA content in IGW-3262 under the DD treatment was similar to WD and DD treatments in Drysdale.
Table 3.2 Leaf water potential ($\Psi_{\text{leaf}}$), stomatal conductance ($g_s$), leaf transpiration rate and net photosynthesis rate of four wheat genotypes at second harvest (170-190 °Cd after anthesis) that were well-watered (WW) or watering withheld from one (WD) or both (DD) halves of the split root system from anthesis. Values are the means of five replicates. LSD values are at 95% level of significance. Photosynthesis was zero in DD treatment.

<table>
<thead>
<tr>
<th></th>
<th>Leaf water potential (MPa)</th>
<th>Stomatal conductance (mmol H$_2$O m$^{-2}$ s$^{-1}$)</th>
<th>Transpiration (mmol H$_2$O m$^{-2}$ s$^{-1}$)</th>
<th>Photosynthesis (μmol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>WD</td>
<td>DD</td>
<td>WW</td>
</tr>
<tr>
<td>IGW-3119</td>
<td>-1.42</td>
<td>-1.68</td>
<td>-3.32</td>
<td>386</td>
</tr>
<tr>
<td>Drysdale</td>
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<td>-2.71</td>
<td>376</td>
</tr>
<tr>
<td>Wyalkatchem</td>
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<td>358</td>
</tr>
<tr>
<td>IGW-3262</td>
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<td>-1.36</td>
<td>-2.80</td>
<td>389</td>
</tr>
<tr>
<td>LSD (genotype)</td>
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<td></td>
<td>43</td>
</tr>
<tr>
<td>LSD (treatment)</td>
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<td></td>
<td></td>
<td>37</td>
</tr>
<tr>
<td>LSD (genotype x</td>
<td>0.43</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

LSD values are at 95% level of significance.
Figure 3.3 Mean leaf ABA concentration in the cultivar Drysdale and the line IGW-3262 at 170 °Cd and 190 °Cd respectively after withholding water from one half (WD) or both halves of the split root system (DD) compared with both halves well-watered (WW). Error bars represent ± SEM, n = 5. Interaction for genotype x treatment was significant, P = 0.001. LSD for the interaction was 230.86 ng g⁻¹ and different letters indicate significant differences between genotypes.

3.3.3 Plant growth

At anthesis, Wyalkatchem had up to two-fold more tillers and green leaf area than the other three genotypes (P < 0.0001; Figure 3.4a, b). Shoot biomass also differed among genotypes at anthesis (P = 0.004), where Wyalkatchem had the largest shoot biomass (Figure 3.4c).

At second harvest, green leaf area of Wyalkatchem WW plants was largest (Table 3.3). Withholding water from one or both halves of the root system significantly reduced leaf area (P = 0.001). Leaf area of plants under the WD treatment was 30% less in Wyalkatchem and 20% less in Drysdale compared with the leaf area of plants under the WW treatment (Table 3.3). Both IGW lines under WD treatment had similar leaf areas to that of plants under the WW treatment. Leaves of plants under the DD treatment had begun to senesce and the leaf net photosynthetic rate was close to zero, so leaf area was not measured.
Tiller production increased in Drysdale and IGW-3119 plants under the WW treatment after anthesis (Figure 3.4a and Table 3.3). However, Wyalkatchem had 35% fewer tillers at second harvest compared with at anthesis, whereas the number of tillers per plant in IGW-3262 was unchanged. Withholding water from one (WD) or both halves of the root system (DD) did not affect tiller number in Wyalkatchem compared with well-watered plants.
Chapter 3 – Stomatal regulation and leaf ABA

(WW) (P > 0.05; Table 3.3). In the other three genotypes, tiller number was reduced by 36% in Drysdale, 16% in IGW-3262 and 13% in IGW-3119 at second harvest. The reduction in tiller number under the DD treatment was similar to WD conditions in Drysdale, IGW-3262 and IGW-3119.

Table 3.3 Green leaf area and tiller number of four wheat genotypes grown in split pots. Plants were well-watered until maturity (WW) or watering was withheld from one (WD) or both (DD) halves of the split root system from anthesis. Second harvest was done at 190 °Cd for IGW-3262 and 170 °Cd for other three genotypes. Values presented are means (n = 5). LSD values are at 95% level of significance. Interaction genotype x treatment was not significant (P > 0.25).

<table>
<thead>
<tr>
<th></th>
<th>Green leaf area (m²)</th>
<th>Tiller number (plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Second harvest</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WW</td>
<td>WD</td>
</tr>
<tr>
<td>IGW-3119</td>
<td>0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>Drysdale</td>
<td>0.14</td>
<td>0.11</td>
</tr>
<tr>
<td>Wyalkatchem</td>
<td>0.20</td>
<td>0.14</td>
</tr>
<tr>
<td>IGW-3262</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>LSD (genotype)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>LSD (treatment)</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

Shoot biomass of all genotypes increased from anthesis to maturity when both halves of the split root system were well-watered (WW) (Figure 3.4c, Table 3.4). Wyalkatchem had the lowest shoot biomass at final harvest of 55 g plant⁻¹, while the other three genotypes averaged 65 g plant⁻¹. Biomass accumulation at second harvest was not affected in IGW-3119 when water was withheld from one half of the root system (WD) while the other genotypes had significant reductions in biomass (P < 0.001; Table 3.4). Withholding water from both halves of the split root system (DD) further reduced shoot biomass at second harvest in all genotypes. At final harvest, shoot biomass was significantly lower in all four genotypes (P = 0.002), especially under the DD treatment which were reduced by 55% in Wyalkatchem to 70% in IGW-3262.
Table 3.4 Shoot biomass of four wheat genotypes that were well-watered until maturity (WW) or watering withheld from one (WD) or both (DD) halves of the split root system from anthesis. The values are means of five replicates. LSD values are at 95% level of significance. Interaction genotype x treatment was not significant at second harvest (P = 0.7) and final harvest (P = 0.06).

<table>
<thead>
<tr>
<th>Shoot biomass (g plant⁻¹)</th>
<th>Second harvest</th>
<th>Final harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>WD</td>
</tr>
<tr>
<td>IGW-3119</td>
<td>26.6</td>
<td>25.3</td>
</tr>
<tr>
<td>Drysdale</td>
<td>27.2</td>
<td>25.1</td>
</tr>
<tr>
<td>Wyalkatchem</td>
<td>28.9</td>
<td>23.9</td>
</tr>
<tr>
<td>IGW-3262</td>
<td>21.8</td>
<td>17.7</td>
</tr>
<tr>
<td>LSD (genotype)</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>LSD (treatment)</td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>

3.3.4 Root growth

Root biomass in each pot half was equal at anthesis, just before withholding water (P = 0.46; Figure 3.5a).

![Figure 3.5](image)

Figure 3.5 Root distribution between two halves of the split root system. (a) Root biomass measured at anthesis (just before withholding water), 170–190 thermal days after withholding water from one half of root systems (second harvest) and at final harvest. (b) Root length (m plant⁻¹) and specific root length (SRL) (m g⁻¹) measured at second harvest. Data from all genotypes were combined. Error bars represent ± SEM; n = 20; * represents significant difference between two pot compartments.
Root biomass distribution between the two compartments was similar in all treatments in all genotypes at second and final harvest (P = 0.5). Hence the root biomass in both pot halves was combined. Total root biomass (both pot halves combined) varied among genotypes. The line IGW-3119 had the smallest root biomass and Wyalkatchem the largest root biomass at anthesis and second harvest under WW conditions (Table 3.5). At second harvest, 170–190 °C days after watering was withheld from one half of the split root system (WD), total root biomass decreased in Drysdale, Wyalkatchem and IGW-3262, but not when watering was withheld from both halves of the root system (DD). Root biomass was unaffected by the watering treatments in IGW-3119 (Table 3.5). At final harvest, root biomass was not affected by watering treatment in Drysdale and IGW-3262, but was reduced in IGW-3119 and Wyalkatchem by similar amounts regardless of whether water was withheld from one or both sides of the pots (Table 3.5).

Table 3.5 Root biomass per plant at second and final harvest of four wheat genotypes that were well-watered (WW) until maturity or watering withheld from one (WD) or both (DD) halves of the split root system from anthesis. Values represent means (n = 5). LSD values are at 95% level of significance.

<table>
<thead>
<tr>
<th>Root biomass (g plant⁻¹)</th>
<th>Second harvest</th>
<th>Maturity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>WD</td>
</tr>
<tr>
<td>IGW-3119</td>
<td>1.16</td>
<td>1.24</td>
</tr>
<tr>
<td>Drysdale</td>
<td>1.46</td>
<td>1.17</td>
</tr>
<tr>
<td>Wyalkatchem</td>
<td>1.78</td>
<td>1.32</td>
</tr>
<tr>
<td>IGW-3262</td>
<td>1.48</td>
<td>0.95</td>
</tr>
<tr>
<td>LSD (genotype)</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>LSD (treatment)</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>LSD (genotype x treatment)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

There were differences in total root length among the genotypes with Wyalkatchem having the longest (623 m) and Drysdale the shortest (291 m) (Table 3.6). There was a significant interaction between genotype and watering treatment (P = 0.002) because of contrasting
responses when water was withheld from one or both halves of the root system compared with when both halves of the root system were watered (WW). Total root length per plant in IGW-3119 was 23% greater than the other genotypes when water was withheld from one (WD) or both halves of the root system (DD). Root length in Drysdale under the WD treatment was similar to that under the WW treatment, but under the DD treatment it increased by 38%. In contrast, root length was decreased by 55% in Wyalkatchem and 43% in IGW-3262 in the WD treatment, but the reduction was negligible in the DD treatment (Table 3.6).

Table 3.6 Total root length per plant and specific root length at second harvest. Plants were grown in split pots that were well-watered (WW) until maturity or watering withheld from one (WD) or both (DD) halves of the split root system from anthesis. Values are means (n = 5). LSD values are at 95% level of significance.

<table>
<thead>
<tr>
<th></th>
<th>Total root length (m)</th>
<th>Specific root length (m g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>WD</td>
</tr>
<tr>
<td>IGW-3119</td>
<td>341</td>
<td>419</td>
</tr>
<tr>
<td>Drysdale</td>
<td>291</td>
<td>308</td>
</tr>
<tr>
<td>Wyalkatchem</td>
<td>623</td>
<td>281</td>
</tr>
<tr>
<td>IGW-3262</td>
<td>458</td>
<td>260</td>
</tr>
<tr>
<td>LSD (genotype)</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>LSD (treatment)</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>LSD (genotype x treatment)</td>
<td>133</td>
<td></td>
</tr>
</tbody>
</table>

Specific root length (SRL) was similar among genotypes under the WW treatment except in Drysdale in which SRL was one-third lower than the other genotypes (Table 3.6). There was a significant interaction between genotype and watering treatment for SRL (P < 0.05) as Drysdale and IGW-3119 produced more fine roots in the treatments where water was withheld after anthesis, increasing root length per unit of weight by 32% and 18% respectively in both the WD and DD treatments, whereas SRL of Wyalkatchem and IGW-3262 was not affected by watering treatment (Table 3.6). SRL in each compartment varied.
with treatment, with more root length in wet compartment (P = 0.017; Figure 3.5b) in all genotypes.

### 3.3.5 Yield and yield components

There was a significant interaction between genotype x treatment on grain yield (P = 0.015). Wyalkatchem under the WW treatment had the lowest grain yield of about 29 g plant\(^{-1}\), while Drysdale and IGW lines had similar yields of about 36 g plant\(^{-1}\). Grain yield under the WD treatment decreased by 16% in IGW-3119, 17% in Wyalkatchem, 20% in Drysdale and 26% in IGW-3262 (Table 3.7; P < 0.01). Under the DD treatment, grain yield in all genotypes was only 10% of that under the WW treatment.

There was significant interaction effect of genotype x treatment for spikes per plant (P < 0.001). The number of spikes per plant varied from 14 in Drysdale to 21 in IGW-3262 (Table 3.7). The number of spikes in IGW-3262 decreased by 29% and 50%, respectively under the WD and DD treatments. The number of spikes per plant did not change in Wyalkatchem under both WD and DD treatments. The spike number in IGW-3119 and Drysdale was not affected under the WD treatment, but decreased by 21% and 26% respectively under the DD treatment. Treatments in which water was withheld from one half (WD) and both halves of the root system (DD) did not change the number of spikelets per spike (P = 0.21; data not shown).

The number of grains per spike varied across the genotypes with Drysdale having the greatest number across all treatments followed by IGW-3262 (P < 0.0001; Table 3.7). The line IGW-3119 and cultivar Drysdale increased the number of grains per spike under the WD treatment compared with WW treatment by 6% (Table 3.7; P < 0.0001), whereas there was no change in IGW-3119 and Wyalkatchem. Under the DD treatment, there were 15 to 20% fewer grains per spike in all genotypes.
1000-grain weight under the WW treatment was highest in IGW-3119 (51 g) and lowest in IGW-3262 (40 g). Drysdale and Wyalkatchem had similar 1000-grain weights of 47 g under the WW treatment. Withholding water from one half of the root system (WD) reduced 1000-grain weight by 20% in IGW-3119, 12% in Drysdale and IGW-3262 and 7% in Wyalkatchem (P = 0.005). All genotypes had similar 1000-grain weights under the DD treatment. Wyalkatchem had lowest harvest index of 0.53 among WW plants (P = 0.003; Table 3.7). Harvest index increased in Drysdale and IGW-3119 under the WD treatment but did not change in Wyalkatchem and IGW-3262. Under the DD treatment, all genotypes had lower harvest index compared with WW treatment. Drysdale and IGW-3262 had higher harvest index under DD treatment (P < 0.0001).
Table 3.7 Yield components and yield of four wheat genotypes that were well-watered until maturity (WW) or watering withheld from anthesis from one (WD) or both (DD) halves of the split root system. Values are means (n = 5) and LSD is at the 95% level of significance.

<table>
<thead>
<tr>
<th></th>
<th>Spikes per plant</th>
<th>Grains per spike</th>
<th>Thousand grain weight (g)</th>
<th>Grain yield (g plant(^{-1}))</th>
<th>Harvest Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>WD</td>
<td>DD</td>
<td>WW</td>
<td>WD</td>
</tr>
<tr>
<td>IGW-3119</td>
<td>17.4</td>
<td>17.0</td>
<td>13.8</td>
<td>39.7</td>
<td>42.4</td>
</tr>
<tr>
<td>Drysdale</td>
<td>14.0</td>
<td>12.2</td>
<td>10.4</td>
<td>52.3</td>
<td>55.6</td>
</tr>
<tr>
<td>Wyalkatchem</td>
<td>16.6</td>
<td>15.0</td>
<td>14.6</td>
<td>35.9</td>
<td>37.9</td>
</tr>
<tr>
<td>IGW-3262</td>
<td>20.5</td>
<td>14.6</td>
<td>10.2</td>
<td>46.8</td>
<td>47.7</td>
</tr>
</tbody>
</table>

LSD (genotype) 1.6 3.3 2.3 1.8 0.03
LSD (treatment) 1.4 2.9 2.0 1.6 0.02
LSD (genotype x treatment) 2.8 NS 4.1 3.1 NS
3.4 Discussion

Stomatal regulation in wheat in response to water deficit is reported to be closely related to increased leaf ABA concentration following a reduction in leaf water potential (Quarrie and Lister, 1983; Henson et al., 1989b). Contrasting responses in $g_s$ and leaf ABA concentration, as well as contrasting responses in $\Psi_{\text{leaf}}$ were observed in this study when one half (WD) or both halves of the split root system (DD) of wheat were exposed to drying soil. Stomatal conductance of the cultivars Drysdale and Wyalkatchem under the WD treatment declined with $\Psi_{\text{leaf}}$ and increased leaf ABA (only measured in Drysdale). On the other hand, IGW lines reduced $g_s$ under the WD treatment without decline in $\Psi_{\text{leaf}}$ or increase in leaf ABA. A lack of correlation between leaf ABA concentration and $g_s$ has been observed in maize (Zhang and Davies, 1990a; Tardieu et al., 1992b), despite ABA in the xylem sap correlating well with $g_s$ (Tardieu et al., 1992b). The results with IGW-3262 indicate that bulk leaf ABA is not the main driver of $g_s$ and probably does not reflect the ABA level acting on stomata in some wheat genotypes.

The contrasting responses in $\Psi_{\text{leaf}}$ when watering was withheld from one half of the split root system (WD) and the corresponding changes in stomata and leaf ABA indicate that the root system of the IGW lines detected the drying soil and produced a signal that triggered partial stomatal closure to prevent water loss and maintain leaf water balance. This root-derived signal could have been ABA (Hartung et al., 2002; Slovik et al., 1995), hydraulic (Comstock, 2002; Christmann et al., 2007), due to change in pH (Hartung et al., 1988; Sobeih et al., 2004) or change in the concentration of another phytohormone (Acharya and Assmann, 2009). Because gas exchange and $\Psi_{\text{leaf}}$ were only measured at the second harvest, we do not know if Drysdale and Wyalkatchem also started to close stomata before any noticeable change in $\Psi_{\text{leaf}}$ or leaf ABA. However, other studies have observed that Drysdale tends to reduce transpiration rate only after soil water decreases considerably
Chapter 3 – Stomatal regulation and leaf ABA

(Schoppach and Sadok, 2012). Hence, Drysdale roots seem to be less sensitive to drying soil, but ABA increases in leaves in response to decreasing $\Psi_{\text{leaf}}$ (Hartung et al., 2002; Westgate et al., 1996), which ultimately caused closure of stomata. It therefore appears that stomatal responses in wheat under terminal drought occur in two stages: primarily in response to a root-derived signal and secondarily in response to a leaf-derived signal. Two-stage stomatal response to soil dryness is consistent with the observations in wheat by Xiong et al. (2006) and Du et al. (2013).

Wheat genotypes differed in their ability to maintain $\Psi_{\text{leaf}}$ when water was withheld from one half of the split root system (WD). Genotypic differences in the strategies to maintain leaf water status have been reported in species like grapes (Schultz, 2003), wherein isohydric varieties regulate stomata to maintain $\Psi_{\text{leaf}}$ and anisohydric varieties allow $\Psi_{\text{leaf}}$ to decline under soil water deficits (Tardieu et al., 1996; McDowell et al., 2008). Lack of soil moisture measurements makes it difficult to ensure that soil moisture status in all genotypes was similar, especially because the leaf area varied between genotypes. Genotypes reached anthesis on different days (Table 3.1) and hence, watering treatment also commenced on different days. However, vapour pressure deficit (VPD) during the period between anthesis and second harvest was $1.18 \pm 0.03$ kPa for all genotypes, indicating similar driving force for transpiration. IGW-3262 reached permanent wilting stage by 190 °Cd while in all other genotypes it was 170 °Cd. Wyalkatchem plants might have dried the soil more quickly than IGW-3262 due to its larger leaf area and root biomass. But this does not appear to be the case with Drysdale since its leaf area and root biomass was similar to IGW-3262 at anthesis and when gas exchange measurements were made. Without the split root system and given the above details, IGW-3262 and Drysdale would be expected to have similar patterns in water use. Comparing the $\Psi_{\text{leaf}}$ of Drysdale and IGW-3262 WD plants with WW plants, IGW-3262 would be considered isohydric and
the cultivar Drysdale as anisohydric. Contrasting iso- and anisohydric behaviour has been observed previously in wheat genotypes, but under osmotic stress (Gallé et al., 2013).

Genotypic difference in the maintenance of $\Psi_{\text{leaf}}$ in WD plants despite having similar leaf area, transpiration rate and VPD level indicates difference in their root level capability to supply water to meet the transpiration demand. The wet half of the Drysdale root system with access to sufficient water was unable to maintain the water status of the shoot, whereas IGW-3262 roots in the wet half of the root system supplied sufficient water to maintain shoot water status. Iso- and anisohydric behaviour is regulated by stomatal control (McDowell et al., 2008) and/or hydraulic conductance (Schultz, 2003). Hence, we propose that isohydric genotypes adapt in response to root signals, maintaining leaf hydration possibly through root hydraulic conductance, which sustains stomatal regulation. However, as the soil drying intensifies, $\Psi_{\text{leaf}}$ declines inducing stomatal closure through increased leaf ABA synthesis. In contrast, $\Psi_{\text{leaf}}$ in anisohydric genotypes decreases either because their root system is less sensitive to soil water potential to initiate stomatal closure and/or their stomatal sensitivity to ABA probably depends on tissue water potential (Tardieu and Davies, 1992).

Drysdale increased SRL when watering was withheld from one half (WD) and both halves of the root system (DD) in response to decreasing soil moisture. An increase in root length with no increase in root biomass under the DD treatment suggests that the difference in SRL was due to production of thin roots and not due to root shrinkage (Huck et al., 1970). Some wheat genotypes are known to produce fine roots under water stress conditions (Manske and Vlek, 2002). Thin roots potentially increased the surface area per unit root volume, resulting in more effective water absorption per unit mass. More thin roots (high SRL) produced in wet compartments than in dry compartments (Figure 3.5) indicate a compensatory adjustment in all genotypes to supplement water supply to shoot. High
specific root length and small root diameters are traits associated with drought resistance (Comas et al., 2013). However, thinner roots in wheat are also associated with lower root hydraulic conductivity because of narrower metaxylem vessels (Schoppach et al., 2014), which is considered beneficial for crops growing in environments with predominantly stored soil moisture (Richards and Passioura, 1989).

When water was withheld from one half of the split root system, reductions of 16–26% in grain yield occurred in all genotypes following 15–70% reduction in net photosynthetic rate, despite sufficient available water in the other half of the split pot. This suggests that the whole root system is needed after anthesis to fully meet the wheat plants demand for water and support grain production. All genotypes had root to shoot biomass ratio of 0.06 ± 0.003 under all treatments, which is considerably lower than the value reported in field-grown wheat (Siddique et al., 1990a). This may be due to lower soil volume in the pot than in field. Due to low soil volume, soil water in the wet half may have rapidly exhausted exposing the plant to water deficit for a short period before the next watering leading to yield reduction.

This study also demonstrates that there is large genotypic variation in the impact of partial soil drying on wheat yield. The line IGW-3262, which maintained leaf water status and gas exchange better than the other genotypes in response to drying signals had the highest yield reduction (25%) mainly due to producing fewer and smaller grains when watering was withheld from one half of the split root system. This is in disagreement with the findings of Westgate et al. (1996) that shoot water status had a positive effect on grain yield. Although the hydrated part of IGW-3262’s root system appears to be able to compensate better than the other genotypes in maintaining leaf hydration under partial soil drying the superior gas exchange did not prevent reductions in shoot biomass to support grain development. In contrast, Drysdale’s grain yield was 6% greater than IGW-3262 under
partial soil water deficit despite Drysdale having the lowest instantaneous leaf photosynthetic rate. A large reduction in net photosynthetic rate compared with the reduction in $g_s$ occurs in aging wheat leaves (Atkinson et al., 1989). Interestingly, this does not translate to grain yield decline; possibly photosynthesis in ear parts of Drysdale contributed more towards grain development (Araus et al., 1993) or the plant partitioned more assimilates to grains (Blum, 1998). Harvest index was slightly higher in the WD than in the WW treatment in Drysdale indicating that this cultivar remobilised more assimilates to the grain. This is in agreement with the studies reported by Zhang et al. (2008). The higher leaf ABA concentration in Drysdale compared with IGW-3262 under the WD treatment, might have also enhanced accumulation of water soluble carbohydrates in the stem and its remobilisation to grains (Yang et al., 2000; Travaglia et al., 2007). Wheat genotypes selected for high leaf ABA accumulation under drought had higher grain yield than low ABA accumulation types (Innes et al., 1984).

### 3.5 Conclusions

Wheat genotypes can have isohydric and anisohydric behaviour under water deficit. Stomata of isohydric genotypes seem to respond to an unknown signal, partially closing stomata. Under severe water deficit (DD treatment), increased leaf ABA is related to decreased leaf water potential. In contrast, stomatal response in anisohydric genotypes initiates with a drop in leaf water status and increase in leaf ABA concentration. Genotypic differences in synthesis of root ABA or other signals in response to water deficit is likely and needs to be considered in future studies. Some genotypes also responded morphologically to limited water supply by producing thin roots to draw water more slowly. The physiological response, however, does not explain the difference in grain yield among genotypes when watering was withheld from one half of the split root system (WD).
4 Root biomass in the drying upper layer of the soil profile affects the initial stomatal response of wheat under terminal drought

4.1 Introduction

Stomatal closure in response to soil water deficit is modulated by abscisic acid (ABA) (Zhang and Davies, 1987; Dodd, 2005), which accumulates in the leaves of some wheat genotypes when exposed to soil dryness (Henson et al., 1989b). Production of ABA in response to drought differs among wheat genotypes (Quarrie, 1980; Quarrie and Henson, 1981). In Chapter 3, the cultivar Drysdale and the breeding line IGW-3262 showed contrasting leaf ABA accumulation when half of their root systems (split vertically) were exposed to terminal drought conditions (Saradadevi et al., 2014). In that study, withholding water from one side of the root system reduced stomatal conductance by 20% in IGW-3262 without any noticeable increase in leaf ABA concentration or change in leaf water potential and by 35% in Drysdale, but the reduction was accompanied by an increase in leaf ABA concentration and a decline in leaf water potential. IGW-3262 was tentatively designated isohydric and Drysdale anisohydric, and their contrasting stomatal responses were speculated to be associated with differences in their root system characteristics to detect and signal soil drying.

Wheat root systems have two root types, seminal and nodal (Manske and Vlek, 2002), and their distribution and architecture vary with genotype (Siddique et al., 1990a; Liao et al., 2006; Manschadi et al., 2006; Palta et al., 2011). ABA accumulation in barley leaves increases with the proportion of the root system exposed to drying soil (Martin-Vertedor and Dodd, 2011). Genotypes, Drysdale and IGW-3262 differed in accumulating ABA in the leaves when only one half of the vertically split root system was exposed to drying soil, while IGW-3262 did not (Chapter 3). Root distribution to the wet and dry half of the pot
was similar in these genotypes. However, they may have different root density in the top layer of the drying half of the pot. It is hypothesized that wheat genotypes with high root density in the drying upper soil layers accumulate more ABA in their leaves, serving as an early signalling mechanism to reduce stomatal conductance and conserve water well before the entire root zone is depleted of water. A segmented-root system experiment that separated the upper and lower soil layers was conducted to test whether the observed difference in leaf ABA accumulation between Drysdale and IGW-3262 is related to differences in root density distribution exposed to drying soil.

4.2 Materials and methods

4.2.1 Plant material and growing conditions

Wheat genotypes Drysdale and IGW-3262 were selected for their contrasting physiological response and ABA production under terminal drought conditions (Saradadevi et al., 2014). The two genotypes were grown in pots in an evaporative-cooled glasshouse at The University of Western Australia, Perth, WA (31° 93’S, 115° 83’E) from June to October 2012. The mean minimum and maximum temperatures in the glasshouse during the experiment were 10.9 ± 0.2 °C and 25.6 ± 0.2 °C, respectively. Mean minimum and maximum relative humidity was 32 ± 5% and 59 ± 6%, respectively. The vapour pressure deficit (VPD) in the glasshouse fluctuated between 1.5 kPa and 2.5 kPa during the post-anthesis period when gas-exchange measurements were taken (Figure 4.1).
Figure 4.1 Mean vapour pressure deficit (VPD) in the glasshouse during the times (11.30 am to 1.30 pm) when gas exchange measurements were conducted from anthesis (0 °Cd) in the cultivar Drysdale and breeding line IGW-3262. Each data point is the mean of nine measurements, recorded at 15 minute intervals.

4.2.2 Segmented pots

Segmented pots were designed to split the wheat root system horizontally into two parts, top and bottom, to maintain the deeper bottom part of the root system in wet soil while allowing the shallower top part to dry (Figure 4.2a). The segmented pots were similar to those described by Gallardo et al. (1994) and Abdelhamid et al. (2011). Briefly, 0.7 m long and 0.24 m diameter polyvinyl chloride (PVC) columns were cut horizontally into two equal halves and re-mounted on top of each other using a PVC collar and sealed to connect the two segments. The top and bottom segments were hydraulically isolated by a wax-coated paper layer supported by plastic wire mesh placed between the two halves before re-mounting and after filling the bottom segment with soil. Preliminary studies demonstrated that the wax layer did not inhibit wheat root growth to the other segment (Figure 4.2b). To aerate the soil in the bottom segment, a perforated tube was positioned to run diagonally from the top of the segment to the bottom (Figure 4.2c). A tubing ring was placed at the top of the bottom segment (below the wax layer) for irrigation (Figure 4.2c).
Chapter 4 – Root distribution and stomatal regulation

Figure 4.2 Diagram of the specially-designed segmented pots used to expose upper and bottom parts of the wheat root system to water deficit from anthesis (a). A wax layer hydraulically separated the two segments to prevent the flow of water and nutrients between the segments, but allowed root penetration (b). Perforated tubes for aeration (transparent tube that runs diagonally down the pot segment) and irrigation (black ring) were installed in the bottom segment to prevent hypoxic conditions and maintain soil moisture contents at required levels, respectively (c).

Both segments of each pot were filled with a reddish-brown sandy clay loam soil mixed with coarse sand as used in Chapter 3. The soil was packed first in the bottom segment and was allowed to settle by slow, repeated hand watering and refilled to prevent the development of hollow spaces between the wax layer and soil over time. This resulted in more compact soil in the bottom segment (1.65 Mg m\(^{-3}\)) than in the top segment (1.49 Mg m\(^{-3}\)). The top segment was filled with soil, leaving a 5 cm space at the top to facilitate watering and to add plastic beads to prevent evaporation from the soil surface. Uniform-sized seeds were germinated in Petri dishes on moist filter paper at room temperature for three days. Three uniform, germinated seedlings were planted in each pot when roots were 0.5 to 1 cm in length. Slow release fertiliser pellets (Osmocote\textsuperscript{®}, N:P:K 13:1.5:4.9) were
mixed into the top 0.1 m of soil in each segment (25 g each) before the two segments were assembled. Two weeks after planting, a water soluble fertiliser with Mg, Cu, Zn, Mo, S and other micronutrients was applied. The soil water content of each segment was monitored separately by inserting a 0.22 m long soil moisture probe (CS620 Hydrosense, Campbell Scientific Inc., Logan, UT, USA) through the horizontal holes in the middle part of each segment (Figure 4.2a). The soil moisture probes were previously calibrated using the same soil–sand mixture to convert the probe reading from percentage volumetric content to gravimetric soil moisture content. The pots were watered on alternate days to maintain the soil water content close to 90% of pot soil water holding capacity until plants were at 50% anthesis—Z65, Zadoks’ growth scale for cereals (Zadoks et al., 1974). Terminal drought was then induced by withholding watering from two-thirds of the pots. Anthesis for each genotype was determined when anthers had emerged from the main stem of 50% of the plants.

4.2.3 Treatments

At 50% anthesis in each genotype, the pots were divided into three groups of ten pots. In the first group, both segments of each pot were well-watered (WW). In the second group, water was withheld from the top segment in each pot (DW), and in the third group, water was withheld from both segments of each pot (DD). Wet soil segments were watered by hand every alternate day to maintain soil water content close to 90% of pot soil capacity based on moisture probe readings. To prevent water loss from the soil through evaporation, the soil surface in the top segment of each pot was covered with a 3 cm layer of white plastic beads.

4.2.4 Measurements

Leaf water potential ($\Psi_{\text{leaf}}$), stomatal conductance ($g_s$), transpiration and leaf net photosynthesis rate were measured on the flag leaf of the different tillers of two plants in
each of five replicate pots in both genotypes. Measurements were made between 10.30 am and 1.30 pm on days with clear sky. Rates of leaf net photosynthesis, \( g_s \) and transpiration were measured with a LI-COR gas-exchange system (LI-6400, LI-COR Biosciences, Nebraska, USA) with LED light source on the leaf chamber. In the LI-COR cuvette, CO\(_2\) concentration was set to 380 µmol mol\(^{-1}\) and LED light intensity 1000 µmol m\(^{-2}\) s\(^{-1}\), which is the average saturation intensity for photosynthesis in wheat (Austin, 1990). Immediately after these measurements were made, \( \Psi_{\text{leaf}} \) was measured using a Scholander-type pressure chamber (Model 1000, PMS Instrument Co., Oregon, USA). The flag leaf was loosely covered with a plastic sheath before excision and during the measurement to avoid evaporation (Turner, 1988). After \( \Psi_{\text{leaf}} \) was measured, the flag leaf was wrapped in aluminium foil, snap frozen in liquid nitrogen and stored at −80 °C for ABA analysis.

4.2.5 Sampling

Shoot and root biomass were measured three times during the experiment at: (1) 50% anthesis, before terminal drought was induced, (2) grain filling, when leaf water potential in the DW treatment fell to −2 MPa, which occurred at 270 °Cd in IGW-3262 and 210 °Cd in Drysdale after withholding water from anthesis and (3) physiological maturity, when 90% of the flag leaves had turned yellow and glumes had lost their green colour in 50% of the plants for each genotype (Hanft and Wych, 1982). Pots in which leaves were removed for \( \Psi_{\text{leaf}} \) and ABA measurements were harvested at grain filling. On each occasion, the number of tillers per plant was recorded; shoots were then cut from the roots at the crown. Green leaf area was measured at anthesis and grain filling using a portable leaf area meter (LI-3000, LI-COR Biosciences, Nebraska, USA). Shoots and leaves were separately dried in an oven at 60 °C for 48 hours and then weighed. Immediately after harvesting the shoots, both pot segments were separated and the roots in each segment recovered from the soil by repeated washing and sieving on a 1.4-mm sieve to produce a clean sample (Liao et al.,
2006; Palta et al., 2007). Roots were then dried in an oven at 60 °C for 72 hours and weighed. Root biomass per plant is the average root biomass of the three plants grown in a pot (one replicate). Root lengths were also measured before drying at the grain filling and physiological maturity harvests. Roots were stained for 30 min with 0.1% (w/v) methylene blue, placed in about 3 mm of water in a glass tray (0.2× 0.3 m) and then untangled with a plastic spatula to minimise overlapping. The glass tray was placed on a flatbed scanner with transparency unit (Epson STD4800). Scanning was done at 400 dpi resolution and the images were analysed for total root length using WinRHIZO Pro 2009b (Regent Instruments Inc., Canada). Specific root length (SRL) in each segment was calculated as root length per unit of root biomass.

At the final harvest, the number of spikes per plant and spikelets per spike were counted. Spikes were separated from shoots, oven dried at 40 °C for one week and threshed by hand. The number and weight of grains per plant were recorded as an average of three plants in a pot (one replicate). Harvest index (HI) was calculated as the ratio of grain yield to shoot biomass.

4.2.6 ABA sampling and analysis

In addition to flag leaves, root samples were collected from the top of each segment, close to the centre of the pot at the grain-filling harvest. The soil was removed with a quick wash, and roots snap frozen in liquid nitrogen and stored at –80 °C until analysis. Analysis was carried out by liquid chromatography/mass spectrometry (LC-MS/MS) as per the protocol described by Speirs et al. (2013) and used in Chapter 3. Briefly, ABA and its catabolites phaseic acid (PA), dihydrophaseic acid (DPA) and glucose ester (ABA-GE) were separated from ground leaf tissue extracts using Phenomenex SPE columns at 40 °C. The separated samples were eluted with acetonitrile and compounds were identified by retention times and multiple reaction monitoring.
4.2.7 Statistical analysis

The experiment was arranged in a randomised complete block design, with replication as the blocking factors. Growth and yield data were analysed with a two-way ANOVA using statistical software R 2.14.0 (R Development Core Team, 2011) followed by the Least Significant Difference (LSD) test for multiple comparisons. The block effect was insignificant in all parameters tested. Growth measured at anthesis was compared using the unpaired t-test. Time series measurement of soil water content was compared through regression analysis. Non-linear regression curve fitting was done using GraphPad Prism 6.03. Best fit was selected after comparing different models based on the extra sum of squares F test. The curves were compared by performing extra sum of squares F test. Thermal time (accumulated daily average temperature, base temperature 0 °C) from anthesis was used for comparison because each genotype reached anthesis on different days and hence gas-exchange measurements were made on different days.

4.3 Results

4.3.1 Phenology

The breeding line IGW-3262 reached anthesis 7 days (107 °Cd) earlier (P < 0.001) and physiological maturity 4 days (84 °Cd) later than Drysdale (Table 4.1). In the DW treatment, time to physiological maturity decreased by 19–20 days (380 to 398 °Cd) compared with the WW treatment. The DD treatment reduced the time to physiological maturity by an additional 13 days (236 °Cd) in IGW-3262 and 9 days (158 °Cd) in Drysdale compared with the DW treatment (Table 4.1).
Table 4.1 Mean number of days after sowing (DAS) to anthesis and physiological maturity in the cultivar Drysdale and breeding line IGW-3262 grown in pots with the top and bottom segments hydraulically separated by a wax layer. Segments were either well-watered (WW), had watering withheld from the top segment only (DW) or from both segments (DD), starting from anthesis. Anthesis was determined when 50% of the genotype was flowering (n = 24) and physiological maturity when 50% of flag leaves had yellowed. Values for days to anthesis are means (n = 24; P < 0.0001). Statistical analysis was not carried out for physiological maturity.

<table>
<thead>
<tr>
<th></th>
<th>Days to anthesis (DAS)</th>
<th>Days to physiological maturity (DAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>DW</td>
</tr>
<tr>
<td>IGW-3262</td>
<td>67</td>
<td>154</td>
</tr>
<tr>
<td>Drysdale</td>
<td>74</td>
<td>150</td>
</tr>
</tbody>
</table>

4.3.2 Soil water content

Soil water content was maintained close to 90% of pot soil capacity in the top and bottom segments of the WW treatment and in the bottom segment of the DW treatment. Soil water content in the top segment of the DW and DD treatments decreased exponentially with thermal time after withholding water from anthesis ($R^2 > 0.8$ for all curves; Figure 4.3a). Drysdale in the DD treatment depleted soil water from the top segment relatively quicker than IGW-3262 under the DW and DD treatments ($P = 0.03$). Withholding watering from both segments in the DD treatment depleted the soil water content in the bottom segment slowly compared with the top segment in both genotypes (Figure 4.3b). The rate of depletion was slower in IGW-3262 than Drysdale.
Chapter 4 – Root distribution and stomatal regulation

Figure 4.3 Soil water content (% pot capacity) in top (a) and bottom (b) segments of the pot in IGW-3262 and Drysdale plants. At anthesis (0 °Cd), watering was withheld from either the top pot segment alone in the DW treatment or from both the top and bottom pot segments in the DD treatment, while both segments in the WW treatment were maintained at 90% pot soil water capacity until maturity. Each data point is the mean of five replicates and errors bars represent SEM. Curves represent best fit to the data (P < 0.05).

4.3.3 Tiller number, leaf area and biomass

At anthesis, Drysdale had almost double the number of tillers, shoot biomass (Table 4.2) and root biomass (Table 4.3) compared with IGW-3262. Drysdale also had 85% more leaf area than IGW-3262 (P = 0.06). In both genotypes, more than 85% of the root biomass was in the top segment of the pot, but Drysdale had more than three times greater root biomass than IGW-3262 in both segments of the pot (Table 4.3).
Table 4.2 Tiller number, leaf area and shoot biomass per plant of the cultivar Drysdale and breeding line IGW-3262 at anthesis, just before the start of drought treatments, and at the second harvest during grain filling. Sampling at grain filling was at 270 °Cd in IGW-3262 and 210 °Cd in Drysdale after withholding water from anthesis. Values at anthesis are mean of five replicates. Values at grain filling are the combined means of all treatments that were not different (P > 0.05), n = 15. Means followed by different letters are significantly different between genotypes at the 95% level of significance.

<table>
<thead>
<tr>
<th></th>
<th>Anthesis</th>
<th></th>
<th>Grain filling</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tiller number</td>
<td>Leaf area (m²)</td>
<td>Shoot biomass (g)</td>
<td>Tiller number</td>
</tr>
<tr>
<td>IGW-3262</td>
<td>5.8ᵇ</td>
<td>0.1ᵃ</td>
<td>4.4ᵇ</td>
<td>8.1ᵃ</td>
</tr>
<tr>
<td>Drysdale</td>
<td>11.0ᵃ</td>
<td>0.1ᵃ</td>
<td>8.6ᵃ</td>
<td>9.4ᵃ</td>
</tr>
<tr>
<td>LSD (genotype)</td>
<td>NS</td>
<td>0.1百度</td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>

At the grain filling harvest, tiller number, leaf area, and root and shoot biomass for each genotype were similar in all treatments (P > 0.05 for each trait). Hence treatment data were combined for each genotype. Both genotypes had the same number of tillers per plant (P = 0.2; Table 4.2), but Drysdale had 70% more leaf area and 30% more shoot biomass than IGW-3262. Drysdale had more root biomass than IGW-3262 in both of the pot segments, 30% more in the top segment and 75% more in the bottom segment (Table 4.3). Both genotypes had similar root lengths in the top segment. In the bottom segment, Drysdale had 60% more root length than IGW-3262. SRL was 25% higher in IGW-3262 than Drysdale in the top segment, but was similar in both genotypes in the bottom segment.
Table 4.3 Root biomass, length and specific root length of the cultivar Drysdale and breeding line IGW-3262 grown in pots horizontally split into two segments with the root system split between top and bottom segments. Measurements were made at anthesis and during grain filling. Sampling at grain filling was at 270 °Cd in IGW-3262 and 210 °Cd in Drysdale after withholding water from anthesis. Values at anthesis are mean of five replicates. Values at grain filling are the combined means of all treatments that were not different (P > 0.05), n = 15. Means followed by different letters are significantly different between genotypes at the 95% level of significance.

<table>
<thead>
<tr>
<th></th>
<th>Anthesis</th>
<th></th>
<th></th>
<th></th>
<th>Grain filling</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Root biomass (g)</td>
<td>Root biomass (g)</td>
<td>Root length (m)</td>
<td>Specific root length (m g⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Top</td>
<td>Bottom</td>
<td>Top</td>
<td>Bottom</td>
<td>Top</td>
<td>Bottom</td>
<td>Top</td>
<td>Bottom</td>
</tr>
<tr>
<td>IGW-3262</td>
<td>0.7ᵇ</td>
<td>0.1ᵇ</td>
<td>1.0ᵇ</td>
<td>0.1ᵇ</td>
<td>232.9ᵃ</td>
<td>44.9ᵇ</td>
<td>213.6ᵃ</td>
<td>340.7ᵃ</td>
</tr>
<tr>
<td>Drysdale</td>
<td>2.2ᵃ</td>
<td>0.3ᵃ</td>
<td>1.4ᵃ</td>
<td>0.2ᵃ</td>
<td>203.7ᵃ</td>
<td>72.4ᵃ</td>
<td>170.2ᵇ</td>
<td>316.9ᵃ</td>
</tr>
<tr>
<td>LSD (genotype)</td>
<td>0.3</td>
<td>0.07</td>
<td>NS</td>
<td>23.8</td>
<td>32.0</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
At the final harvest, both genotypes had a similar number of tillers under the WW treatment. Withholding water from the top segment and from both segments reduced tiller number by on average 46% (P < 0.0001; Table 4.4). Drysdale had more shoot biomass (35.4 g per plant) (P < 0.0001) than IGW-3262 (26.8 g per plant). Final shoot biomass under the DW and DD treatments was reduced by an average of 55% in both genotypes. Both genotypes had similar root biomass, root length and SRL under all watering treatments at final harvest (P > 0.05 for each trait). The top segment had 1.65 g of root biomass and 239 m root length per plant while the bottom segment had 0.27 g of biomass and 64 m of length. SRL in the top and bottom segments was therefore, 150 and 235 m g⁻¹, respectively.

Table 4.4 Tiller number and shoot biomass at maturity of the cultivar Drysdale and breeding line IGW-3262 grown in pots horizontally split into two segments with the root system split between top and bottom segments. From 50% anthesis, both segments were either well-watered (WW), had watering withheld from the top segment only (DW) or from both segments (DD). Values are the mean, n = 5. LSD values are at 95% significance. Means followed by different letters are significantly different between genotypes and treatments at the 95% level of significance.

<table>
<thead>
<tr>
<th></th>
<th>Tillers per plant</th>
<th>Shoot biomass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IGW-3262</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>12.9 a</td>
<td>26.8 b</td>
</tr>
<tr>
<td>DW</td>
<td>7.0 b</td>
<td>11.7 d</td>
</tr>
<tr>
<td>DD</td>
<td>5.7 c</td>
<td>13.0 d</td>
</tr>
<tr>
<td><strong>Drysdale</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>12.8 a</td>
<td>35.4 a</td>
</tr>
<tr>
<td>DW</td>
<td>6.6 b</td>
<td>15.6 c</td>
</tr>
<tr>
<td>DD</td>
<td>7.7 b</td>
<td>17.3 c</td>
</tr>
<tr>
<td>LSD (genotype × treatment)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LSD (genotype)</td>
<td>1.00</td>
<td>3.04</td>
</tr>
<tr>
<td>LSD (treatment)</td>
<td>1.23</td>
<td>3.72</td>
</tr>
</tbody>
</table>
4.3.4 Leaf water potential ($\Psi_{\text{leaf}}$), stomatal conductance ($g_s$), transpiration and leaf net photosynthesis rate

When water was withheld from the top segment alone and from both segments, $\Psi_{\text{leaf}}$ decreased with thermal time compared to that of the WW treatment (Figure 4.4a, b). After withholding water from anthesis, IGW-3262 maintained $\Psi_{\text{leaf}}$ as per the WW treatment until 141 °Cd in the DW and 107 °Cd in the DD treatments and then started declining. $\Psi_{\text{leaf}}$ had decreased by 40% in DW and 50% in DD treatment at 207 °Cd. In Drysdale, $\Psi_{\text{leaf}}$ in the DW and DD treatments was lower than the WW treatment as early as 67 °Cd after withholding water from anthesis. $\Psi_{\text{leaf}}$ decreased by similar amounts in both DW and DD treatments, to 50% by 211 °Cd.

In WW plants, $g_s$ varied between 200 and 470 mmol m$^{-2}$ s$^{-1}$ in IGW-3262 and in Drysdale between 220 and 330 mmol m$^{-2}$ s$^{-1}$. $g_s$ declined in both genotypes in the DW and DD treatments, with the reduction observed earlier in Drysdale than in IGW-3262 (Figure 4.4c and d). In Drysdale, $g_s$ in the DW and DD treatments was 40% lower by 67 °Cd compared with the WW treatment. In IGW-3262, $g_s$ reduction was only observed at 141 °Cd in the DD treatment and at 175 °Cd in the DW treatment.

Transpiration rate in the WW treatment fluctuated more in IGW-3262 (Figure 4.4e) than Drysdale with Drysdale showing a slight linear increase with thermal time after anthesis (Figure 4.4f). Transpiration rate was lower in the DW and DD treatments in both genotypes compared with the WW treatment. Similar to the $g_s$ response, transpiration rate in Drysdale was significantly lower in DW and DD treatments at an earlier thermal time compared with IGW-3262.

The net photosynthesis rate under WW conditions varied in IGW-3262 (Figure 4.4g), but remained stable in Drysdale (Figure 4.4h). After withholding post-anthesis watering,
photosynthesis rate declined over thermal time in the DW and DD treatments following the same trends as $g_s$ and transpiration.

Figure 4.4 Change in leaf water potential ($\Psi_{\text{leaf}}$) (a,b), stomatal conductance ($g_s$) (c, d), transpiration rate (e,f) and photosynthesis rate (g, h) in IGW-3262 (left panel) and Drysdale (right panel) over thermal time after anthesis. At anthesis (0 °Cd), water was withheld from the top pot segment in the DW treatment or from both top and bottom pot segments in the DD treatment, while both pot segments in the WW treatment and bottom segments in the DW treatment were maintained at 90% pot capacity until maturity. Each data point is the mean of five replicates, except for leaf water potential where the mean was of three or four replicates. Error bars represent SEM.
4.3.5 Abscisic acid and its metabolites in leaf and root tissues

In IGW-3262, leaf ABA content varied over time, and the pattern of variation was similar across treatments (Fig 4.5a). Under WW conditions, leaf ABA in IGW-3262 varied between 96 and 310 ng g\(^{-1}\). Leaf ABA content had increased in the DW and DD treatments by 175 °Cd compared with the WW treatment. In Drysdale, this increase was observed at 99 °Cd in the DD treatment. At 130 °Cd, leaf ABA in the DW and DD treatments was 90 to 100% higher than in the WW treatment. \(g_s\) was negatively related to leaf ABA content in a curvilinear relationship that was best fit in both genotypes, but the goodness of the fit was greater in Drysdale (\(R^2 = 0.54\)) compared with IGW-3262 (\(R^2 = 0.12\)) (Figure 4.5c and d).

![Graphs showing changes in leaf abscisic acid (ABA) concentration with thermal time after anthesis (a, b) and its relationship with stomatal conductance (\(g_s\)) (c, d) in IGW-3262 (left panel) and Drysdale (right panel). At anthesis (0 °Cd), water was withheld from the top pot segment (DW) or from both top and bottom pot segments (DD), while both pot segments in WW and bottom segments in DW were maintained at 90% pot capacity until maturity. Each data point is the mean of five replicates; error bars are SEM.](image)

**Figure 4.5** Changes in leaf abscisic acid (ABA) concentration with thermal time after anthesis (a, b) and its relationship with stomatal conductance (\(g_s\)) (c, d) in IGW-3262 (left panel) and Drysdale (right panel). At anthesis (0 °Cd), water was withheld from the top pot segment (DW) or from both top and bottom pot segments (DD), while both pot segments in WW and bottom segments in DW were maintained at 90% pot capacity until maturity. Each data point is the mean of five replicates; error bars are SEM.
Under WW conditions, the molar ratio between PA and ABA (PA:ABA), which indicates the balance between ABA degradation to PA and ABA synthesis, remained constant with thermal time in IGW-3262 at around 0.4, but fluctuated between 0.1 and 0.4 in Drysdale (Figure 4.6a, b). PA:ABA was higher in the DW and DD treatments than in WW, with a steep increase in IGW-3262 at 175 °Cd. DPA was beyond detection in most of the samples analysed, so no data is shown. The molar ratio between ABA-GE and ABA (ABA-GE:ABA), the balance between ABA degraded by conjugation and ABA synthesis, was constant with thermal time in Drysdale at around 0.1 and similar in all treatments (Figure 4.6d), while in IGW-3262, it fluctuated between 0.1 and 0.4 in all treatments (Figure 4.6c).

Figure 4.6 Changes in the ratio of molar concentrations of phaseic acid (PA) to ABA (a, b) and glucose ester of ABA (ABA-GE) to ABA (c, d) in leaf tissues of IGW-3262 (left panel) and Drysdale (right panel). At anthesis (0 °Cd), water was withheld from the top pot segment (DW) or from both top and bottom pot segments (DD) while both pot segments in WW and bottom segments in DW were maintained at 90% pot capacity until maturity. Each data point is the mean of three to five replicates; error bars are SEM.

Root ABA content, measured during the grain-filling harvest, was 70.9 ± 8.1 ng g$^{-1}$ in both genotypes under all treatments (P > 0.05). ABA-GE and PA in the root tissue was 26.7 ±
3.4 and 31.0 ± 3.4 ng g⁻¹, respectively, in both genotypes and did not change under water stress conditions (P > 0.05).

### 4.3.6 Yield and yield components

There was a significant interaction effect of genotype × treatment for grain yield (P = 0.0009). Under WW conditions, Drysdale had 50% more grain yield than IGW-3262 (Table 4.5). When water was withheld from the top segment, grain yield decreased by 63% in IGW-3262 and 51% in Drysdale. When water was withheld from both segments, grain yield decreased by 60–65% in both genotypes.

**Table 4.5** Grain yield and yield components of the cultivar Drysdale and breeding line IGW-3262 grown in pots horizontally split into two segments with the root system split between top and bottom segments. From 50% anthesis, both segments were either well-watered (WW), had watering withheld from the top segment only (DW) or from both segments (DD). Values are the mean, n = 5. LSD values are at the 95% significance. Means followed by different letters are significantly different between genotypes and treatments at the 95% level of significance.

<table>
<thead>
<tr>
<th></th>
<th>Grain yield (g)</th>
<th>Spikes per plant</th>
<th>Grains per spike</th>
<th>1000 grain weight (g)</th>
<th>Harvest Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IGW-3262</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>14.5ᵇ</td>
<td>9.1ᵃ</td>
<td>55ᵃ</td>
<td>36.8ᵇ</td>
<td>0.57ᵃ</td>
</tr>
<tr>
<td>DW</td>
<td>5.3ᵉ</td>
<td>5.2ᵇ</td>
<td>47ᵃ</td>
<td>25.0ᵈ</td>
<td>0.53ᵇ</td>
</tr>
<tr>
<td>DD</td>
<td>5.8ᵈᵉ</td>
<td>5.0ᵇ</td>
<td>52ᵃ</td>
<td>22.7ᵉ</td>
<td>0.44ᶜ</td>
</tr>
<tr>
<td><strong>Drysdale</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>21.5ᵃ</td>
<td>9.6ᵃ</td>
<td>50ᵃ</td>
<td>43.6ᵃ</td>
<td>0.58ᵃ</td>
</tr>
<tr>
<td>DW</td>
<td>10.6ᶜ</td>
<td>5.1ᵇ</td>
<td>48ᵃ</td>
<td>32.0ᶜ</td>
<td>0.50ᵇ</td>
</tr>
<tr>
<td>DD</td>
<td>7.3ᵈ</td>
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<td>47ᵃ</td>
<td>24.2ᵉ</td>
<td>0.43ᶜ</td>
</tr>
<tr>
<td>LSD (genotype × treatment)</td>
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<td>NS</td>
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<tr>
<td>LSD (genotype)</td>
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<td>NS</td>
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<td>NS</td>
</tr>
<tr>
<td>LSD (treatment)</td>
<td>1.34</td>
<td>1.34</td>
<td>NS</td>
<td>4.03</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Under WW conditions, both Drysdale and IGW-3262 had nine spikes per plant (P = 0.069). Spike number was decreased in both genotypes by 45% in the DW and DD treatments.
Both genotypes averaged 50 grains per spike under all watering treatments ($P > 0.05$). The 1000-grain weight was 18% higher in Drysdale than in IGW-3262 under WW conditions (Table 4.5), decreased by 25% in Drysdale and 35% in IGW-3262 in the DW treatment and was similar in both genotypes in the DD treatment.

Harvest index (HI) was 0.57 in both genotypes under WW conditions ($P = 0.76$) and decreased in both genotypes to 0.53 in the DW treatment and 0.43 in the DD treatment (Table 4.5).

### 4.4 Discussion

The wheat cultivar Drysdale and the breeding line IGW-3262 differ in how they regulate stomatal conductance when exposed to terminal drought conditions. When watering was withheld from either the top segment (DW) or both segments (DD) of the pot, Drysdale started closing stomata earlier than IGW-3262. Drysdale had a larger leaf area than IGW-3262, indicating higher transpiration demand, and more root biomass, particularly in the top segment of the pot, which resulted in slightly earlier and faster soil water depletion than IGW-3262.

The soil water content in the top soil segment after a marked reduction in $g_s$ in Drysdale was around 60% of pot capacity, while it was only 50% in IGW-3262. Considerably higher root biomass in the drying top segment in Drysdale may have served as a signal generation source resulting in relatively higher leaf ABA concentration and subsequent stomatal closure. Reduced $g_s$ associated with an increase in leaf ABA concentration has been observed in barley leaves when a larger proportion of root system was exposed to drying soil (Martin-Vertedor and Dodd, 2011).
Increased ABA synthesis in root tissues exposed to drying soil is reportedly the signal for stomatal closure (Hartung et al., 2002; Speirs et al., 2013). However, it does not seem possible that increased synthesis of ABA in root tissues in both genotypes served as the drying signal to initiate stomatal closure in this study, because increased ABA concentration in root tissues was not detected in response to soil water deficit. This is supported by the findings of Christmann et al. (2005) in Arabidopsis where no increase in root ABA was observed under water stress.

Xylem ABA has better control of stomatal conductance than leaf ABA under water stress in species like maize (Tardieu et al., 1992b). In wheat, xylem sap ABA concentration increased in response to water stress (Munns and King, 1988; Ali et al., 1998), but a clear influence on stomatal conductance has not been established (Atkinson et al., 1989; Ali et al., 1998). In this study, a decline in gs was observed to precede a remarkable increase in leaf ABA in both genotypes similar to that previously observed in maize (Blackman and Davies, 1985; Zhang and Davies, 1990a; Tardieu et al., 1992b). This suggests that xylem ABA may be the driver for the initial gs response to water stress (Zhang and Davies, 1990a; Tardieu et al., 1992b). It is not clear if ABA synthesised in the root is transported away from the root through xylem (Slovik et al., 1995; Hartung et al., 2002). An alternative to root ABA as the source of xylem ABA, is possibly that leaf ABA was translocated to the roots through phloem (Zeevaart and Boyer, 1984; Slovik et al., 1995; Kudoyarova et al., 2011) and contributed to high xylem ABA (Hartung et al., 2002). Alkalisation of xylem sap due to reduced NO₃ uptake may also have contributed to the increased translocation of leaf ABA into the xylem and leaf apoplast in the absence of de novo synthesis (Gollan et al., 1992; Schurr et al., 1992; Wilkinson et al., 2007).

The high sensitivity of Drysdale stomata to drying soil signals was not expected since the anisohydric behaviour described in Chapter 3 was thought to be due to a slow stomatal
response to drying soil (Saradadevi et al., 2014). In Chapter 3, root system was split vertically into two halves and distribution to both wet and dry compartments was equal at the time of imposing water stress. The water flow from one-side of the root exposed to wet soil may have diluted the signal concentration generated by the other half of roots in drying soil (Tardieu et al., 1992a). However, in the current study more of the root system was distributed in the top segment. The bulk density of the soil in the bottom segment was higher than in the top segment and probably sufficient to cause soil compaction (Tardieu et al., 1992a). Subsoil compaction is common in the Western Australian wheat belt (Dracup et al., 1992; Chen et al., 2014) and may have restricted root penetration and growth (Hurd, 1968) resulting in less than 20% of the total root biomass distributed in the bottom segment.

In lupin, when the upper half of the root system was exposed to drying soil, the lower half provided sufficient water to the shoot to maintain $\Psi_{\text{leaf}}$ and $g_{\text{s}}$ at similar levels to well-watered plants (Gallardo et al., 1994). In wheat, roots in the deeper layer that comprised only 3% of the total root biomass was sufficient to supply water to the plant when the top soil layer dried (Gregory et al., 1978a; Gregory et al., 1978b). However, in this study, withholding water from the top segment in the DW treatment developed plant water deficit and reduced gas exchange even though sufficient water was available in the bottom segment and is similar to the response of faba bean (Abdelhamid et al., 2011) when soil in the upper half of the root system was dried. Higher soil compaction in the bottom segment may have restricted soil water extraction (Tardieu et al., 1992a). Similar $\Psi_{\text{leaf}}$ and gas exchange in DW and DD treatments supports this assumption. As a result of the soil compaction in the bottom segment, the experimental procedures described here were unable to validate the effect of water flow from the bottom roots in the signal perception in leaves.
The relationship between ABA accumulation in leaves and $g_s$ appears to differ between Drysdale and IGW-3262. In Drysdale, the decline in $g_s$ was negatively associated with increases in ABA content in leaf tissue as observed previously (Saradadevi et al., 2014) and another wheat genotype by Henson et al. (1989b). In IGW-3262, the relationship between leaf ABA and $g_s$ was weaker and so leaf ABA was discounted as the major driver for stomatal regulation (Saradadevi et al., 2014). Leaf ABA varied in IGW-3262 in all watering treatments with sharp declines at 141, 175 and 279 °Cd, indicating that factors other than soil water status were influencing leaf ABA accumulation. Soil compaction can trigger ABA accumulation in the leaves; however it is unlikely to cause fluctuation in the ABA level (Tardieu et al., 1992a). Hence, the variations in leaf ABA concentration may possibly be caused by environmental factors such as humidity. Exposure to high humidity has been reported to reduce leaf ABA concentration in Arabidopsis (Okamoto et al., 2009).

ABA level in tissues can be lowered by two alternate pathways: hydrolysis to PA and conjugation with glucose to form the ester, ABA-GE (Cutler and Krochko, 1999). Expression of genes favouring breakdown of ABA to PA has been observed under low VPD conditions in grape vine (Speirs et al., 2013). However, the PA:ABA ratio in IGW-3262 was relatively constant apart from a peak at 175 °Cd in DW and DD treatments corresponding to the decline in leaf ABA. When converted to PA, further metabolism to DPA and other catabolites (Cowan and Railton, 1987; Zhou et al., 2004) suggest that the PA pool alone does not represent ABA catabolism. However, the PA: ABA ratio was higher in IGW-3262 than Drysdale indicating that more ABA is metabolised in IGW-3262. Alternatively, further catabolism of PA to other products may be slow in IGW-3262. Interestingly, ABA-GE: ABA ratio fluctuated in IGW-3262, and the pattern of fluctuation was similar to the variation in leaf ABA level, with a higher ratio corresponding to a dip in ABA level. So, it can be assumed that the reduction in leaf ABA was due to its increased catabolism to ABA-GE in addition to being converted to PA under DW and DD treatments.
and mainly by ABA-GE under WW treatment. Conjugation of ABA with glucose to form ABA-GE is considered an irreversible process to sequester ABA from the cells (Zeevaart and Creelman, 1988). Our results suggest that variation in leaf ABA and stomatal behaviour observed between Drysdale and IGW-3262 are related to the difference in ABA catabolism. However, with limited data available, a clear conclusion cannot be drawn at this time.

Grain yield reduced in Drysdale and IGW-3262 consistent with the yield reduction observed in field grown wheat under terminal drought (Dias de Oliveira et al., 2013). A similar yield reduction was reported in faba bean when soil in the top segment dried while that in the bottom segment had sufficient water (Abdelhamid et al., 2011). A reduction in the number of fertile tillers (tillers with spikes) and poor grain filling, reflected in the lower 1000-grain weight, contributed to this yield reduction. The size of the reduction in grain yield due to terminal drought in Drysdale was less than IGW-3262 in the DW treatment, despite root restrictions for extracting water from the bottom segment. Early stomatal closure in response to the drying top soil may have resulted in more efficient water use in Drysdale than in IGW-3262 (Sinclair et al., 1984). Conversely, later stomatal closure in IGW-3262 enabled photosynthesis rates to be maintained, which likely supported the extra vegetative growth, tiller production and root growth that occurred in IGW-3262 after anthesis. However, some of these late-formed tillers did not produce a spike or the small ear failed to fill grains. The yield of Drysdale in the DW treatment was 45% more than in the DD treatments indicating that roots in the bottom segment made a significant contribution to the plant water supply. Higher yield in DW than DD treatment was surprising since physiological response of Drysdale under DW treatment within 300 °Cd after withholding water from anthesis was not better than DD treatment. The drying signals from the top roots may have declined or stopped when water flow from those roots eventually reduced as soil water was exhausted (Dodd et al., 2008a). Alternatively, the
hydraulic conductivity of roots in the bottom segment may have increased in due course to meet transpiration demand (Vysotskaya et al., 2003; Vysotskaya et al., 2004). In contrast, IGW-3262 had similar yields in the DW than DD treatments indicating that bottom roots were not effective in extracting water as in Drysdale. This suggests that Drysdale bottom roots have better capability to extract water compared to those of IGW-3262, even under conditions where root growth was restricted. The severe yield reduction and lower HI in Drysdale and IGW-3262 under the DD treatment arose from a dramatic reduction in 1000-grain weight reflecting the impact terminal drought has on the grain yield of wheat.

### 4.5 Conclusions

Initial stomatal closure in the wheat cultivar Drysdale and breeding line IGW-3262 in response to terminal drought is linked to differences in root density in the upper drying soil layer and is probably associated with signal strength translocated to the shoot. Drysdale had more root biomass in the drying top soil layer and initiated stomatal closure earlier than IGW-3262. Xylem ABA appears to mediate initial stomatal regulation in both genotypes. Both genotypes clearly differed in their stomatal response to soil water deficit, and also in their relationship between gs and leaf ABA. The mechanism of this variation is unclear, but presumably is due to ABA metabolism. In addition to early stomatal closure, Drysdale seems to be better at extracting water from the bottom segment resulting in a narrower yield gap than IGW-3262.
5 Stomatal conductance in response to exogenous application of abscisic acid to the flag leaves of two wheat genotypes

5.1 Introduction

The regulation of stomatal conductance ($g_s$) to minimise water loss through transpiration in response to drying soil conserves soil water and facilitates prolonged availability of soil water to plants. Closing stomata at the end of the season is not generally acceptable as it reduces photosynthesis. However, keeping stomata open could rapidly exhaust soil water. For every extra millimetre of water that is used after anthesis, wheat yield increases. Hence, reducing $g_s$ to prolong availability of soil water during grain filling can be rewarding in wheat production, especially under terminal drought where crops rely on stored soil moisture. As the soil dries, the root system senses the drying soil and produces and transport soil drying signals to the shoot. Increased abscisic acid (ABA) concentration in the leaves leads to stomatal closure in wheat (Henson et al., 1989b), but the relationship between leaf ABA and $g_s$ varies with genotypes. In the vertical split root system (Chapter 3) and horizontally segmented root study (Chapter 4), there was a strong negative association between leaf ABA and $g_s$ in the cultivar Drysdale, while the relationship was weak in the line IGW-3262. In Chapter 4, a reduction in $g_s$ preceded an increase in leaf ABA suggesting xylem ABA was the possible signal that initiated stomatal closure in both genotypes (Zhang and Davies, 1990a; Tardieu et al., 1992b). The source of ABA contributing to xylem ABA is not clear since there was no increase in ABA in the root tissue in response to drying the soil. This does not necessarily imply that the root is not the source of increased xylem ABA because synthesised ABA may be transported away from the root before any apparent increase in concentration. Alternatively, leaf ABA translocated to xylem through phloem can be a contributor of xylem ABA. In addition, signals other
than ABA, such as xylem pH (Hartung et al., 1988; Wilkinson and Davies, 1997) and hydraulic signals (Comstock, 2002) can act as an early signal to initiate stomatal closure.

In addition to the fluctuations in $g_s$ observed in IGW-3262 in Chapter 4, leaf ABA also fluctuated irrespective of drought treatment. However, fluctuations in $g_s$ were not in tune with the fluctuation in leaf ABA resulting in a weak relationship between $g_s$ and leaf ABA in IGW-3262. If stomatal closure is driven by xylem ABA concentration then the fluctuation in $g_s$ in IGW-3262 should be in response to differences in xylem ABA concentration. Thus, one would expect the stomata in IGW-3262 to be more sensitive to xylem ABA concentration. On the contrary, stomata in Drysdale closed earlier than IGW-3262 when soil water was depleted from the top soil layer. Drysdale also demonstrated a strong relationship between leaf ABA and $g_s$. Therefore, it appears that these genotypes differ in stomatal sensitivity to xylem ABA, if xylem ABA is the driving force of stomatal regulation in both genotypes.

To test whether stomata are more sensitive to xylem ABA in IGW-3262 compared with Drysdale, a leaf feeding experiment was designed. Stomatal conductance was measured on detached leaves with their cut ends bathed in solutions containing different concentrations of ABA. A leaf feeding experiment was chosen rather than quantifying ABA concentration in xylem exudates from roots because collection of sufficient xylem sap volume for ABA analysis is difficult (Dodd and Davies, 1996) in wheat and barley (Cramer and Lewis, 1993; Munns et al., 1993). In wheat, xylem sap has been collected in limited studies, mainly from seedlings, either by pressurizing the whole root system in a pressure chamber (Munns et al., 1993) or by exudation (Ali et al., 1998; Vysotskaya et al., 2003). Neither of these methods produced adequate sap volume for analysis in drought stressed wheat in preliminary tests for this study. Additional objectives were to identify if stomatal sensitivity varies with (i) soil water status or (ii) pH of xylem sap.
5.2 Materials and methods

The cultivar Drysdale and the breeding line IGW-3262 were grown in pots in an evaporative-cooled glasshouse at UWA from June to October 2013. Ninety six plastic pots of 0.24 m diameter were filled to a depth of 0.30 m with soil. The soil was a 3:2 mixture of soil and sand as described in detail in Chapter 3. The pot soil water capacity (PSWC) of each pot was determined by weighing the pot after 48 hours of saturation. Two pre-germinated seeds were planted in each pot. Pots were maintained at 90% PSWC until anthesis to prevent anaerobic conditions due to the clayey texture of soil. At anthesis, watering was withheld from two-thirds of the pots, while the remaining pots were maintained at 90% PSWC. In order to avoid fluctuations in $g_s$ due to atmospheric conditions such as VPD as observed in Chapter 4, plants were moved to a controlled environment room the day before measurements. The environmental conditions were set similar to average conditions in the glasshouse and light intensity under which similar bioassays were conducted (Munns and King, 1988; Zhang and Davies, 1991; Munns, 1992; Munns et al., 1993).

The walk-in controlled environment room (interior capacity 16 m$^3$) was maintained at 22/12 °C day/night temperature, 65 ± 5% relative humidity (RH) and 12 hour daylight intensity of 300 µmol m$^{-2}$ s$^{-1}$ photosynthetically active radiation at canopy level. Lighting was provided through metal halide lamps (Eye Lighting, Multi-Ace, MF 1000W BUH, Tokyo, Japan) and was regulated diurnally. Air temperature was regulated by refrigerative cooling and electric element heaters. Required humidity levels were maintained using steam injection and dehumidification (Munters, Sweden). Airflow was distributed downward uniformly.
5.2.1 Measurements

Measurements were taken when half of the pots from which water was withheld reached 50% PSWC and the other half 25% PSWC. Stomatal conductance ($g_s$), instantaneous transpiration rate and photosynthetic rate in flag leaves on the main stem from both genotypes under well-watered and water deficit conditions were measured. Measurements were made with a LI-COR gas exchange system (LI-6400, LI-COR Biosciences, Nebraska, USA) with LED light source on the leaf chamber, between 10 and 11 am. CO$_2$ concentration in the LI-COR cuvette was set to 380 ppm and LED light intensity 300 μmol m$^{-2}$s$^{-1}$, equivalent to the leaf-level light intensity in the controlled environment room. Leaf water potential ($\Psi_{leaf}$) was measured on the penultimate leaf on the main stem using a Scholander pressure chamber (model 1000, PMS Instrument Co., Oregon, USA). Immediately after measuring $g_s$, flag leaves were detached from the main stem by cutting under water to avoid embolism. The cut ends of the detached leaves were placed in preweighed plastic vials containing 10 ml of solution with or without ABA. ABA solutions were prepared using reagent grade (±)-ABA (Sigma-Aldrich, St Louis, USA) dissolved in methanol and diluted to concentrations of $10^{-6}$ M, $10^{-7}$ M or $10^{-8}$ M. To test sensitivity changes in response to pH, ABA solutions were buffered to pH 6 and 7 using 10 mM solutions of the organic buffers MES (pH 6) or HEPES (pH 7), respectively. These buffers have been used previously in exogenous ABA feeding trials with different plant species (Guerrero and Mullet, 1986; Lee et al., 1990; Trejo et al., 1993; Schwartz et al., 1994). To avoid direct light, vials were covered with aluminium foil and sealed with insulation tape. Four leaves for each ABA concentration were used as replications. One replication was measured per day for each genotype and same procedure was repeated for remaining three replicates on consecutive days (Figure 5.1). The vials with leaves were kept at leaf-level height of intact plants in the controlled environment room for 2 hours. After 2 hours, $g_s$ of the leaves was measured, final weight of vials was recorded and $\Psi_{leaf}$ was measured. Soon
after the measurements, the outline of the leaf above the aluminium foil was traced onto paper and leaf area was measured using a portable leaf area meter (LI-3000, LI-COR Biosciences, Nebraska, USA) before snap freezing the leaf in liquid nitrogen for ABA analysis. Transpiration rate was calculated as the weight loss of the vials per unit leaf area per second, assuming that rate was constant over the 2 h duration. Transpiration rate and \( g_s \) of leaves fed with different ABA concentrations were expressed as ratios of the leaves of well-watered plants in solution without ABA at the corresponding pH. Flux of ABA into the leaves was also calculated as the concentration of ABA flowing through a cross section of leaf per second from the transpiration rate and initial concentration of ABA in each solution (Jia et al., 1996).

**Figure 5.1** A diagrammatic representation of the experimental design to test the effect of abscisic acid (ABA) concentration and xylem pH on stomatal conductance of flag leaves excised from the main stem of IGW or Drysdale. The cut end of leaves was inserted into vials containing ABA solutions of differing concentrations and pH. To avoid direct light, vials were covered with aluminium foil and sealed with insulation tape. The design was replicated for each genotype and each soil moisture status before leaf excision.
5.2.2 ABA analysis

ABA analysis was carried out by liquid chromatography/mass spectrometry (LC-MS/MS) as per the protocol of Speirs et al. (2013) and described in Chapter 3. Briefly, ABA and its catabolites, phaseic acid (PA), dihydrophaseic acid (DPA) and glucose ester (ABA-GE), were separated from ground leaf tissue extracts using Phenomenex SPE columns at 40 °C. The separated samples were eluted with acetonitrile and compounds were identified by retention times and multiple reaction monitoring.

5.2.3 Statistical analysis

Gas exchange measurements in intact flag leaves just before detachment were analysed by performing two-way ANOVA using the statistical software R 2.14.0 (R Development Core Team, 2011). Mean values of flag leaves of two plants grown per pot were considered a single replication and there were 16 replications for each treatment.

For the ABA feeding part of the study, a three way ANOVA was carried out separately for two genotypes with PSWC treatments, ABA concentrations and pH as main factors. Separate analysis was carried out for each genotype as gs in IGW-3262 was reduced even in control solution (See section 5.3.2). Ratio data were transformed logarithmically, wherever necessary, to satisfy the requirements of normal distribution and equality of variance for ANOVA. For leaf ABA, a three way ANOVA was carried out with genotypes, PSWC treatments and ABA concentrations as main factors. Multiple comparisons were done by LSD test.
5.3 Results

5.3.1 Impact of soil water deficit on $\Psi_{\text{leaf}}, g_s$, transpiration and photosynthesis of leaves in intact plants

Leaf water potential ($\Psi_{\text{leaf}}$) of penultimate leaves on the main stem in both genotypes was –0.8 ± 0.14 MPa in 90% and 50% PSWC in both genotypes, but decreased to –2.2 MPa in IGW and –2.5 MPa in Drysdale under the 25% PSWC treatment (P < 0.05; Table 5.1).

$g_s$ of intact leaves had significant interaction effect of genotype × watering treatment (P = 0.03). At 90% PSWC, $g_s$ was 16% higher in IGW-3262 than in Drysdale (Table 5.1). At 50% PSWC, $g_s$ decreased by 28% in Drysdale, but did not change in IGW-3262. The $g_s$ of both genotypes decreased by 87% at 25% PSWC.

Table 5.1 Stomatal conductance, instantaneous transpiration rate, net photosynthesis and leaf water potential ($\Psi_{\text{leaf}}$) of the line IGW-3262 and cultivar Drysdale grown in pots with 90%, 50% and 25% of pot soil water holding capacity (PSWC) after withholding water from anthesis. $g_s$, transpiration rate and photosynthesis rate were measured on the flag leaf of the main stem and $\Psi_{\text{leaf}}$ was measured on the penultimate leaf. Values are means of 16 replicates. LSD values are at 95% level of significance.

<table>
<thead>
<tr>
<th></th>
<th>Stomatal conductance (mmol m⁻² s⁻¹)</th>
<th>Transpiration rate (mmol m⁻² s⁻¹)</th>
<th>Net photosynthesis (µmol m⁻² s⁻¹)</th>
<th>Leaf water potential (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IGW-3262</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90% PSWC</td>
<td>376.7</td>
<td>2.8</td>
<td>11.1</td>
<td>–0.7</td>
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<tr>
<td>50% PSWC</td>
<td>339.4</td>
<td>2.6</td>
<td>10.1</td>
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<td>25% PSWC</td>
<td>50.0</td>
<td>0.6</td>
<td>3.2</td>
<td>–2.2</td>
</tr>
<tr>
<td><strong>Drysdale</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90% PSWC</td>
<td>324.0</td>
<td>2.4</td>
<td>9.9</td>
<td>–0.7</td>
</tr>
<tr>
<td>50% PSWC</td>
<td>234.7</td>
<td>1.9</td>
<td>9.2</td>
<td>–0.8</td>
</tr>
<tr>
<td>25% PSWC</td>
<td>41.1</td>
<td>0.7</td>
<td>2.2</td>
<td>–2.5</td>
</tr>
<tr>
<td>LSD (genotype x treatment)</td>
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<td>NS</td>
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<tr>
<td>LSD (genotype)</td>
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<td>0.2</td>
<td>0.5</td>
<td>NS</td>
</tr>
<tr>
<td>LSD (treatment)</td>
<td>35.1</td>
<td>0.3</td>
<td>0.6</td>
<td>0.1</td>
</tr>
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</table>
Transpiration rate had a significant interaction effect for genotype × PSWC treatment (P = 0.01). At 90% PSWC, the transpiration rate was similar in both genotypes. At 50% PSWC, transpiration rate declined by 20% in Drysdale, but did not change in IGW-3262. Transpiration rate decreased by 80% in IGW-3262 and by 70% in Drysdale resulting in similar transpiration rates at 25% PSWC (Table 5.1).

The main effects of genotype and treatment were significant for photosynthetic rate (P < 0.0001). IGW-3262 had a 10% higher photosynthetic rate than Drysdale at both 90% and 50% PSWC (Table 5.1). In both genotypes, net photosynthetic rate decreased by 10% at 50% PSWC and 75% at 25% PSWC.

5.3.2 g	extsubscript{s} and transpiration in detached leaves fed with different concentrations of ABA

When intact leaves were cut and inserted in MES (pH 6) or HEPES (pH 7) solution with no ABA for 2 hours, g	extsubscript{s} was similar to that of intact leaves in Drysdale (P = 0.95; Figure 5.2). However in IGW-3262, g	extsubscript{s} in both MES and HEPES solution without ABA was about 50% lower than that in intact leaves (P = 0.01).

![Figure 5.2 Stomatal conductance (g	extsubscript{s}) of (a) IGW-3262 and (b) Drysdale in flag leaves on the main stem just before excision (intact leaves) and detached leaves two hours after insertion into buffer solutions, MES (pH 6) and HEPES (pH 7), without ABA. Error bars represent SEM, n = 4. Different letters indicate significant differences between intact and detached leaves.](image-url)
Chapter 5 – Stomatal response to exogenous ABA

The $\Psi_{\text{leaf}}$ of all leaves after 2 hours of feeding ABA solutions was around $-0.3$ MPa in 90% PSWC and 50% PSWC plants and $-0.25$ MPa in 25% PSWC plants ($P = 0.01$; Figure 5.3), regardless of genotype ($P = 0.1$), pH ($P = 0.9$) or ABA solution ($P = 0.5$). Stomatal conductance ratio ($Cr$) was calculated as the ratio of $g_s$ of leaves in different ABA solutions to that of the corresponding control. Leaves of well-watered plants (90% PSWC) in MES (pH 6) and HEPES (pH 7) solutions without ABA (0ABA) were considered controls. Similarly, ratio of transpiration rate from the whole leaf ($Tr$) during 2 hours of ABA feeding was calculated.

**Figure 5.3** Leaf water potential ($\Psi_{\text{leaf}}$) of detached flag leaves in wheat plants grown in pots with 90%, 50% and 25% of pot soil water holding capacity (PSWC) and after feeding with ABA solutions of different concentration for 2 h. Data for genotypes and different ABA solutions were combined. Error bars represent SEM, $n = 4$. Different letters indicate significant differences between PSWC treatments.

pH had a significant effect on Cr in IGW-3262 ($P < 0.01$), but not in Drysdale ($P > 0.4$). Cr in IGW-3262 had a significant interactive effect for PSWC treatment $\times$ ABA concentration ($P = 0.004$)., but was not significant, in Drysdale ($P = 0.8$). However the main effects were significant for Drysdale. In IGW-3262, 90% PSWC leaves fed with $10^{-7}$ or $10^{-8}$ M ABA solution at pH 6 had Cr similar to that of the control (0 ABA), but Cr decreased when fed with $10^{-6}$ M ABA solution (Table 5.2). $10^{-7}$ M ABA solution decreased Cr in 90% PSWC at pH 7 and in 50% PSWC leaves at pH 6 and 7. In Drysdale, interactive effect for PSWC
Chapter 5 – Stomatal response to exogenous ABA

treatment × ABA concentration was not significant (P = 0.8). However the main effects were significant. 90% PSWC and 50% PSWC leaves fed with $10^{-8}$ M ABA solution had Cr similar to that of the control, but Cr decreased when fed with $10^{-7}$ M ABA solution. In both genotypes, Cr was lower than control under 25% PSWC and ABA concentration had no the effect on $g_s$ of 25% PSWC leaves.

Table 5.2 Ratios of $g_s$ and transpiration rate of flag leaves detached from plants grown in pots with 90%, 50% and 25% of pot soil water holding capacity (PSWC) fed with ABA solutions of different concentration for 2 hours. Ratios were calculated relative to that of flag leaves of well-watered plants (90% PSWC) fed with solution containing no ABA. ABA solutions were of 0, $10^{-8}$, $10^{-7}$ and $10^{-6}$ M concentrations at pH 6 and 7. The # symbol indicates that values for pH 6 and pH 7 are combined, n = 8. Values for conductance ratio of IGW-3262 are means of 4 replicates. LSD values are at 95% significance. Interaction effects of PSWC treatment x ABA concentrations x pH and ABA concentrations x pH were not significant.

<table>
<thead>
<tr>
<th></th>
<th>Stomatal conductance ratio</th>
<th>Transpiration ratio #</th>
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<tbody>
<tr>
<td></td>
<td>ABA solutions (M)</td>
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</tr>
<tr>
<td></td>
<td>0</td>
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</tr>
<tr>
<td>IGW-3262</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 6</td>
<td></td>
<td></td>
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<tr>
<td>90% PSWC</td>
<td>1.00</td>
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<th>Drysdale</th>
<th>IGW-3262</th>
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<tr>
<td>LSD (treatment x ABA)</td>
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<td>NS</td>
<td>0.21</td>
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<tr>
<td>LSD (treatment x pH)</td>
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<td>LSD (treatment)</td>
<td>0.13</td>
<td>0.20</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>LSD (ABA)</td>
<td>0.15</td>
<td>0.22</td>
<td>NS</td>
<td>0.12</td>
</tr>
<tr>
<td>LSD (pH)</td>
<td>0.10</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
Tr in Drysdale had a significant PSWC treatment × ABA concentration effect (P = 0.04). The ABA concentration of $10^{-8}$ M had no effect on Tr of 90% PSWC leaves (Table 5.2). ABA concentration of $10^{-7}$ and $10^{-6}$ M solutions reduced Tr of 90% and 50% PSWC leaves. Leaves of plants at 50% and 25% PSWC had lower Tr than the control even when fed with 0 M ABA. Tr was similar in all ABA-fed 25% PSWC leaves and was similar to those of 90% PSWC and 50% PSWC leaves fed with $10^{-6}$ M ABA solution. In IGW-3262, Tr was similar in 90% and 50% PSWC leaves fed with different ABA solutions (P > 0.05), but was lower in 25% PSWC leaves (P = 0.04).

Average flux of ABA into the leaf during the two hour of ABA feeding had significant interaction effect of PSWC treatment × ABA concentration (Figure 5.4; P < 0.01) in both genotypes. In both genotypes, the average ABA flux was similar in leaves fed with 0 and $10^{-8}$ M ABA solutions, but significantly higher in $10^{-7}$ and $10^{-6}$ M ABA solutions.

5.3.3 Leaf ABA content as affected by feeding exogenous ABA concentration

Since the pH effect on $g_{s}$ and transpiration was not remarkable in Drysdale and plants at 90% PSWC and 50% PSWC did not show considerable difference in their Cr and Tr responses to feeding similar concentrations of ABA solutions, leaf ABA analysis was done at 90% PSWC and 25% PSWC plants fed with ABA solutions at pH 6.

Leaf ABA content had a significant interactive effect of genotype × PSWC treatment × ABA concentrations (P = 0.02). IGW-3262 and Drysdale had similar ABA contents under all conditions except in 25% PSWC leaves fed with $10^{-6}$ M ABA solution (Figure 5.5). Leaf ABA was similar to the control (0 ABA) in all leaves fed $10^{-8}$ and $10^{-7}$ M ABA solutions in both genotypes. When fed $10^{-6}$ M ABA solution, ABA increased by 590% and 420%, respectively in 90% PSWC and 25% PSWC leaves in IGW-3262 compared to the control (0ABA). In Drysdale, leaf ABA was 870% and 1500% higher than the control in 90% PSWC and 25% PSWC leaves, respectively.
Figure 5.4 Average ABA flux into leaves of IGW-3262 (a) and Drysdale (b) when flag leaves detached from plants grown in pots with 90%, 50% and 25% of pot soil water holding capacity (PSWC) were fed with ABA solutions of different concentration. ABA solutions were of $0$, $10^{-8}$, $10^{-7}$ and $10^{-6}$ M ABA. Error bars are SEM, n = 4.
Figure 5.5 Leaf ABA in (a) IGW-3262 and (b) Drysdale when flag leaves of the main stem of well-watered plants (90% PSWC) and plants at 25% pot water holding capacity (25% PSWC) were fed with ABA solutions of different concentration. ABA solutions were of 0, $10^{-8}$, $10^{-7}$ and $10^{-6}$ M ABA. Error bars are SEM, n = 4.
There was a significant PSWC treatment × ABA concentration interaction effect on phaseic acid (PA) content (P < 0.001), but no significant difference between genotypes (P = 0.6).

PA was similar to the control in 90% PSWC leaves fed with $10^{-7}$ and $10^{-8}$ M ABA solutions, but increased by 250 to 400% when fed $10^{-6}$ M ABA (Table 5.3). PA content in 25% PSWC leaves in all ABA solutions was similar. The ratio of PA to ABA was higher in 25% PSWC leaves than 90% PSWC leaves. The ratio increased with ABA concentration of $10^{-6}$ M, but was similar to the control in $10^{-7}$ and $10^{-8}$ M ABA (Table 5.3).

Table 5.3 Accumulation of ABA metabolite, PA and molar ratio of PA to ABA in the flag leaves of plants were grown in pots with 90%, 50% and 25% of pot soil water holding capacity (PSWC) when fed with ABA solutions of different concentration. ABA solutions were of 0, $10^{-8}$, $10^{-7}$ and $10^{-6}$ M ABA. Values are means of four replicates. LSD values are at 95% level of significance.

<table>
<thead>
<tr>
<th>PA (ng g$^{-1}$)</th>
<th>PA : ABA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABA solutions (M)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>IGW-3262</td>
<td></td>
</tr>
<tr>
<td>90% PSWC</td>
<td>49.8</td>
</tr>
<tr>
<td>25% PSWC</td>
<td>1038.3</td>
</tr>
<tr>
<td>Drysdale</td>
<td></td>
</tr>
<tr>
<td>90% PSWC</td>
<td>34.3</td>
</tr>
<tr>
<td>25% PSWC</td>
<td>886.3</td>
</tr>
<tr>
<td>LSD (genotype x treatment x ABA)</td>
<td>NS</td>
</tr>
<tr>
<td>LSD (genotypes x treatment)</td>
<td>NS</td>
</tr>
<tr>
<td>LSD (genotype x ABA)</td>
<td>NS</td>
</tr>
<tr>
<td>LSD (treatment x ABA)</td>
<td>2.6</td>
</tr>
<tr>
<td>LSD (genotype)</td>
<td>NS</td>
</tr>
<tr>
<td>LSD (treatment)</td>
<td>1.6</td>
</tr>
<tr>
<td>LSD (ABA)</td>
<td>2.0</td>
</tr>
</tbody>
</table>

There was a significant genotype × PSWC treatment (P < 0.001) interaction effect on leaf ABA-GE content. Drysdale had lower ABA-GE content than IGW-3262 at 90% PSWC and 25% PSWC (Table 5.4). ABA-GE increased in the 25% PSWC leaves by 150% in Drysdale and 210% in IGW-3262 compared to that in 90% PSWC leaves. Genotype ×
ABA concentration effect was also significant on leaf ABAGE (P = 0.03). Leaf ABA-GE was similar to the control in leaves fed $10^{-7}$ M ABA, but lower in $10^{-8}$ M ABA in both genotypes. However, when fed $10^{-6}$ M ABA, ABA-GE increased in Drysdale, while it was lower than the control in IGW-3262. The ratio of ABA-GE to ABA was higher in IGW-3262 than Drysdale (P = 0.03) in all treatments (P = 0.2; Table 5.4). The ratio was lower in ABA solutions than the control, with the lowest ratio in $10^{-6}$ M ABA.

**Table 5.4** Accumulation of glucose ester of ABA (ABA-GE) and molar ratio of ABAGE to ABA in the flag leaves of plants grown in pots with 90%, 50% and 25% of pot soil water holding capacity (PSWC) when fed with ABA solutions of different concentration. ABA solutions were of 0, $10^{-8}$, $10^{-7}$ and $10^{-6}$ M ABA. Values are means of four replicates. LSD values are at 95% level of significance.

<table>
<thead>
<tr>
<th></th>
<th>ABA-GE (ng g⁻¹)</th>
<th>ABA-GE : ABA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>$10^{-8}$</td>
</tr>
<tr>
<td><strong>IGW-3262</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90% PSWC</td>
<td>99.0</td>
<td>68.0</td>
</tr>
<tr>
<td>25% PSWC</td>
<td>268.3</td>
<td>252.3</td>
</tr>
<tr>
<td><strong>Drysdale</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90% PSWC</td>
<td>60.7</td>
<td>61.3</td>
</tr>
<tr>
<td>25% PSWC</td>
<td>168.0</td>
<td>143.0</td>
</tr>
</tbody>
</table>

LSD (genotype x treatment x ABA) | NS | NS
LSD (genotype x treatment) | 13.6 | NS
LSD (genotype x ABA) | 19.3 | NS
LSD (treatment x ABA) | NS | NS
LSD (genotype) | 9.6 | 0.15
LSD (treatment) | 9.5 | NS
LSD (ABA) | 13.5 | 0.22

**5.4 Discussion**

Stomatal response of wheat genotypes, Drysdale and IGW-3262 to exogenous ABA application indicates that these genotypes differ in their stomatal sensitivity to ABA concentrations, pH and possibly osmolality of the solution fed.
5.4.1 Exogenous ABA concentration higher than that found in xylem sap of water-stressed wheat plants is required to affect similar stomatal closure.

g_s and transpiration rate of intact flag leaves of IGW-3262 did not decline at 50% PSWC, but declined in Drysdale by 28% confirming the observation in Chapter 4 that Drysdale is more sensitive to soil water deficit than IGW-3262. This reduction in g_s may be in response to increased xylem ABA concentration (Tardieu et al., 1992b). The ABA concentration in the xylem sap of wheat exposed to drought that closed stomata was $10^{-8}$ M ABA (Munns et al., 1993). One would expect that at 50% PSWC leaves of Drysdale to maintain reduced g_s when fed $10^{-8}$ M ABA solution. On the contrary, $10^{-8}$ M ABA solution maintained similar Cr and Tr values to control plants, but a higher concentration of $10^{-7}$ M ABA solution could reduce Cr of 50% PSWC leaves similar to intact leaves. An ABA concentration as high as $10^{-6}$ M was required to reduce stomatal conductance (Cr) and transpiration rate (Tr) of well watered (90% PSWC) and 50% PSWC plants to values similar to that of 25% PSWC plants. This is in accordance with the observations of Munns et al. (1993) on the transpiration effect when exogenous ABA solution was fed to wheat plants.

The lack of reduction in transpiration rate and g_s when plants were fed with concentrations of ABA similar to those found in xylem sap of wheat under water-stress was not due to increased sequestration of ABA to leaf tissues or increased ABA metabolism (Trejo et al., 1993); leaf ABA and the ABA metabolites PA and ABA-GE were not remarkably higher than control in leaves fed with ABA solution of $10^{-8}$ M concentrations. The increased $\Psi_{leaf}$ due to ABA feeding may have reduced the stomatal sensitivity to ABA concentration and altered the g_s response to ABA concentration (Tardieu et al., 1993). Thus, it appears that stomatal closure is a function of ABA concentration in the xylem sap as well as hydration status of the leaves (Tardieu and Davies, 1992). Alternatively, stomatal response may be a function of ABA flux through the xylem (Raschke, 1975). ABA flux in Drysdale in $10^{-8}$ M
ABA solution was similar to the control. As the ABA concentration increased to $10^{-6}$ M, $g_s$ decreased to a level similar to $g_s$ in plants under 25% PSWC. Leaf ABA also increased, even though ABA metabolism increased with increasing concentration of ABA (Jia et al., 1996). Considering the ABA flux of 0.01 nM ABA m$^{-2}$ s$^{-1}$ in leaves fed $10^{-6}$ M solution, and assuming that ABA reaching the leaves was segregated into leaf tissue, the average ABA that could accumulate in leaf tissue is approximately 380 ng g$^{-1}$. Leaf ABA content of flag leaves fed with 0 ABA solution was considered as the initial leaf ABA content before feeding ABA solutions. Thus, the maximum leaf ABA content expected on leaves fed $10^{-6}$ M is around 500 ng g$^{-1}$. However, the leaf ABA content in $10^{-6}$ M ABA-fed leaves was 100% higher than expected. Moreover, this accumulated leaf ABA is above that which is rapidly metabolised to PA and ABA-GE. In species like commelina and maize, ABA fed through xylem rapidly metabolised within 45 minutes of feeding (Jia et al., 1996). It is reasonable, therefore, to assume that elevated leaf ABA content in the flag leaves fed with $10^{-6}$ M ABA resulted from increased leaf ABA synthesis. Increased ABA synthesis, despite $\Psi_{\text{leaf}}$ as high as $-0.3$ MPa, suggests that lower $\Psi_{\text{leaf}}$ to trigger ABA synthesis in leaves (Henson, 1985) is not always essential.

5.4.2 Effect of pH of the solution on $g_s$ and transpiration

Stomata in IGW-3262 appear to be more sensitive to factors other than ABA concentration, including pH of the solution. As the pH of the feeding solution increased from 6 to 7, $g_s$ decreased indicating that there was an effect of pH on $g_s$ in IGW-3262. This is consistent with the findings in maize (Bahrun et al., 2002), Commelina communis (Wilkinson and Davies, 1997) and capsicum (Dodd et al., 2003), where a rise in xylem pH induced stomatal closure. However, a rise in xylem pH alone (without ABA) did not initiate a reduction in transpiration in tomato leaves (Wilkinson et al., 1998). Similarly, there was no reduction in $g_s$ in Drysdale when the pH of the solution increased with or without the presence of ABA.
5.4.3 Possible differences between genotypes in stomatal sensitivity to factors other than xylem ABA concentration and pH

g_s in IGW-3262 under both MES and HEPES solutions without ABA decreased to half of the intact plants while Drysdale maintained high g_s. The high Ψ leaf after feeding the leaves with ABA solutions in comparison with that of intact plants indicates that there were no cavitations or blockage of xylem vessels that caused the reduction in g_s in IGW-3262. The reduction in g_s was not in response to any increase in leaf tissue ABA and for that reason leaf ABA can be excluded as the driving factor for g_s in IGW-3262 (Saradadevi et al. (2014) and Chapter 3). Since the experiment was conducted in controlled environment conditions, this g_s response is unlikely to be associated with environmental effects. Moreover, the g_s response was similar across replications, which was scattered on different days. Thus, it appears that stomata in IGW-3262 are sensitive to other factors besides xylem ABA concentration and pH, probably to the osmolality of the xylem sap (Kelly et al., 2013). In addition to the increase in xylem ABA concentration, significant changes in the composition of xylem sap has been reported in species such as sunflower (Gollan et al., 1992) and maize (Bahrun et al., 2002). Increased concentrations of malate and mannitol—the end products of photosynthesis—in the apoplast are thought to play in closing stomata (Patonnier et al., 1999), possibly due to osmotic changes that regulate stomatal aperture (MacRobbie, 1980). Alternatively, lack of supply of signals such as cytokinin from the root may have restricted stomatal opening in IGW-3262 (Blackman and Davies, 1985).

5.4.4 Evidence for possible differences in ABA metabolism between the cultivar Drysdale and the line IGW-3262

Leaf ABA when fed with $10^{-8}$ and $10^{-7}$ M ABA solutions was similar to the control in both genotypes. As ABA concentration increased to $10^{-6}$ M, leaf ABA increased in both genotypes, more so in Drysdale than IGW-3262. This is consistent with the findings from the split root study described in Chapter 3, where Drysdale increased leaf ABA when half
of the root system was exposed to drying soil. It can be suggested that Drysdale leaves are more sensitive to drying signals from the roots producing leaf ABA much faster than IGW-3262 at similar concentration of xylem ABA. Both genotypes had similar increase in PA in response to increased ABA synthesis indicating similar rates of ABA metabolism via hydrolysis. However, ABA metabolism by conjugation with glucose to form its ester ABA-GE only slightly differs between these two genotypes. When plants were fed with $10^{-6}$ M ABA solution, ABA-GE concentration increased in Drysdale, but decreased in IGW-3262. A similar discrepancy in the relationship between ABA and ABA-GE in IGW-3262 and Drysdale was observed in the study described in Chapter 4.

Conjugation of ABA with glucose to form ABA-GE is usually considered an irreversible process to sequestrate ABA from the cells (Zeevaart and Creelman, 1988). Alternatively, ABA-GE can be hydrolysed by β-glucosidase to yield biologically-active ABA (Hartung et al., 2002; Xu et al., 2012). Polymerisation of β-glucosidase localised in endoplasmic reticulum of *Arabidopsis thaliana* released free ABA into the apoplastic space of leaves from ABA-GE in leaf cells (Lee et al., 2006). The reduction in ABA-GE as ABA increased in IGW-3262 suggests that such hydrolysis to release free ABA happens in IGW-3262. It is not known if such β-glucosidases function in wheat to release free ABA from ABA-GE. However, it has been reported that increases in transpiration inhibitory capability of wheat sap by the polymerisation of small compounds in the sap (Munns et al., 1993) leads to the possibility of β-glucosidases functioning in wheat. Thus, hydroxylation of leaf ABA-GE to raise apoplastic ABA is a possible pathway adopted by IGW-3262 to regulate its stomata, which may explain the weak relationship between leaf ABA and $g_s$ observed in Chapters 3 and 4.
5.4.5 Stomatal reversal to the usual conductance was not possible in the leaves of plants under 25% PSWC when the leaves were fed with 0ABA solution

At 25% PSWC, leaves had 60% lower $g_s$ than the control in all solutions with or without ABA indicating that factors other than ABA concentration in the xylem sap residing within leaves also regulate stomata. Thus, previous exposure to higher ABA or other drying signals seems to alter the sensitivity of stomata to a given ABA concentration. Results also underline the limitations in the reversal of stomatal closure even after 2 hours of rehydration. This is consistent with the stomatal response of cherry leaves where $g_s$ did not return to the usual conductance even after three hours of transferring the leaves to ABA free buffer (Gowing et al., 1993). However, at 50% PSWC leaves which had reduced $g_s$ in intact plants reversed their usual $g_s$ values, similar to the control at that lower $\Psi_{leaf}$. It is expected that at 25% PSWC leaves may have been exposed to a much higher ABA concentration than at 50% PSWC leaves and that the duration of exposure may have been longer. Higher leaf ABA concentrations at 25% PSWC leaves may sustain stomatal closure (Henson et al., 1989b). Reduced $g_s$, despite having similar post-feeding $\Psi_{leaf}$ close to –0.3MPa, implies that stomatal closure is not fully controlled by turgor (Pierce and Raschke, 1980) or leaf water status (Henson, 1985). The presence of other chemicals such as cytokinins may be essential to revert stomatal closure (Blackman and Davies, 1983). Results from the present study suggest that stomatal response is not a direct function of current xylem ABA concentration (Zhang and Davies, 1990a), but other factors such as pH, osmolality of the sap as well as previous exposure to higher ABA concentration may be involved.
5.5 Conclusions

The stomatal response of wheat genotypes, IGW-3262 and Drysdale, to exogenous ABA application indicates that stomata in the line IGW-3262 are sensitive to several factors other than ABA concentration, such as pH and presumably osmolality of the solution. The differences in the relationship between $g_s$ and ABA among the two genotypes may be associated with different ABA metabolism in these two genotypes. ABA concentration equivalent to that found in the xylem sap of water-stressed wheat plants was not sufficient to reduce $g_s$. Instead, a higher concentration was needed to initiate stomatal closure. Similarly, a complete reversal of stomatal closure upon rehydration was not possible in plants at 25% PSWC compared to those under 50% PSWC. Thus, the apparent sensitivity of stomata is not a straight forward relationship with the current xylem ABA concentration, but appears to be interlinked with several other factors such as pH of xylem sap, water status of leaves, leaf ABA concentration and possibly osmolality of xylem sap.
6 The root capacity to uptake water from deep soil layers differs in two wheat genotypes contrasting for stomatal conductance

6.1 Introduction

In the event of terminal drought, soil dries progressively from the shallow to the deeper layers of the soil profile, exposing the shallow part of the root system to dry soil while the deeper part of the root system has some water available to complete the grain filling. Chapter 4 showed that when the root system in the top part of the pot was exposed to drying soil, the cultivar Drysdale initiated stomatal closure earlier and at soil water content higher than the breeding line IGW-3262. These responses were related to Drysdale depleting the soil moisture faster due to greater root biomass in the top soil layer than IGW-3262.

Stomatal behaviour of these two genotypes also differed. Stomata of IGW-3262 were sensitive to unknown factors other than soil water status, as observed in chapter 4. Drysdale stomatal conductance was related to the accumulation of abscisic acid (ABA) in the leaf tissues (Chapters 3 and 4). However, stomatal closure in both genotypes commenced before any remarkable increase in leaf ABA was detected. As there was no increase in the ABA content in root tissues under water deficit conditions, translocation of ABA from the leaves to the root via phloem was postulated as the source of increased ABA in the xylem sap ascending to the shoot. The chances of root ABA being translocated to shoot as xylem ABA without increasing the root ABA content was also not discounted. Sampling the xylem sap to unravel the mechanism behind different stomatal behaviour of these genotypes was not possible because of the great difficulty in obtaining xylem sap from full-grown water-stressed wheat plants (Munns et al., 1993).

Reduced stomatal conductance in response to drying top soil has been reported to have a negative impact on wheat yield (Blum and Johnson, 1993), particularly affecting grain
Chapter 6 – Root capacity to uptake water

filling under terminal drought (Saini and Aspinall, 1981; Kobata et al., 1992; Rajala et al., 2009). Grain filling is reduced mainly because reduced stomatal conductance limits photosynthesis (Henson et al., 1989a; Chaves et al., 2009) and hence, production of current assimilates, the major source of carbon for grain filling (Kobata et al., 1992; Palta et al., 1994; Blum, 1998). The proportion of biomass converted to grain yield is determined mainly by the water used after anthesis (Passioura, 1983). Thus, every extra millimetre of water used during the grain filling can result in a yield advantage (Manschadi et al., 2006; Kirkegaard, 2007). Reduced stomatal conductance limits transpiration and may maintain soil water availability for use during grain filling, resulting in increased water use efficiency (Sinclair et al., 1984). Conservative water use through reduced stomatal conductance may outweigh the negative impact of reduced photosynthesis on yield.

In Chapter 4, it was also shown that grain yield reduced in both Drysdale and IGW-3262 when the top soil dried, but the yield reduction was 21% lower in Drysdale than IGW-3262. The yield difference resulted from a poor grain filling resulting in low 1000—grain weight. The poor grain filling was likely associated with a reduction in water uptake and use as duration of grain filling and subsequently final grain weight was reduced (Gooding et al., 2003). The better yield performance of Drysdale than IGW-3262 under conditions of terminal drought is likely a result of differences in post-anthesis water use and water use efficiency due to differences in availability of soil water at depth. To test this hypothesis, an experiment was conducted in a glasshouse during May to October 2014.

6.2 Materials and methods

6.2.1 Plant materials and growing conditions

Wheat genotypes IGW-3262 and Drysdale were grown in an evaporative-cooled glasshouse at CSIRO Floreat Park, WA (31° 95′ S, 115° 79′ E) from May to October 2014. Plants were grown in polyvinyl chloride pots of 0.15 m diameter and 1.0 m depth filled with the same
Chapter 6 – Root capacity to uptake water

soil described in Chapter 3. Uniform-sized seeds were germinated in Petri dishes on moist filter paper at room temperature for three days. Five pre-germinated seeds were planted per pot on 6th May 2014, and were thinned to three per pot at the three to four leaf stages. At planting, nutrients equivalent to 60 kg N ha⁻¹, 77 kg P ha⁻¹ and 70 kg K ha⁻¹ as a commercial N:P:K fertilizer was mixed in the top 10 cm of the soil in each pot. A water soluble fertiliser with Mg, Cu, Zn, Mo, S and other micronutrients were supplied through irrigation at tillering stage. The pots were weighed for soil water content twice a week and watered to maintain 90% pot water capacity until anthesis when the treatments were applied. A 5 cm layer of plastic beads was uniformly spread on the top of the soil in each pot to prevent losses of soil water through evaporation. Plants were also grown in 12 extra pots for measuring leaf water potential at three different times during the development of the water stress.

6.2.2 Treatments

Terminal drought treatments were induced at anthesis, (Z 65; Zadoks’ growth scale for cereals, (Zadoks et al., 1974), when anthers had emerged from the main stem of 50% of the plants. Terminal drought was induced by withholding water from 28 pots (14 pots per genotype). In 14 of these pots (7 pots per genotype), the soil profile was allowed to dry completely (WS). In the other 14 Pots (7 pots per genotype), a known amount of water was supplied to the bottom 30 cm of the soil profile through an irrigation tube connected to a plastic bottle (Figure 6.1) to keep the bottom 30 cm of the soil profile in the pot at 100% water holding capacity (WB). The drained water in this treatment was continuously collected in a plastic bottle connected to the end of a drainage pipe in each pot and added back to the bottom 30 cm of the soil the following day after weighing. Injecting water through the tubing was started when the pot reached 60% of its total water holding capacity (equivalent to 40% of plant available water assuming that the lower limit of extractable soil water is 10% soil moisture content) after withholding water at anthesis. This occurred at 99
°Cd and 167 °Cd after withholding water at anthesis in Drysdale and IGW-3262, respectively. A third group of 14 pots (7 pots per genotype) were well-watered to maintain the soil at 90% pot water holding capacity (WW) until physiological maturity. Physiological maturity was determined when 90% of the flag leaves had turned yellow and glumes had lost their green colour in 50% of the plants for each genotype (Hanft and Wych, 1982). Out of 7 pots under each treatment per genotype, 4 pots were used for gas exchange and yield measurements and 3 pots for measuring leaf water potential.

Figure 6.1 Diagram of the polyvinyl chloride pots used to apply different watering treatments from anthesis to physiological maturity: WW, well-watered (maintained at 90% of the pot capacity); WB, watering withheld until 60% pot water capacity and then maintained at this level until maturity; WS, watering withheld from anthesis to maturity. Irrigation was applied in the WB treatment by adding water to a side plastic bottle (a) connected to an irrigation tube (b) located 30 cm from the bottom of the soil profile in the pot and drainage was collected in a plastic bottle connected to an outlet at the bottom of the pot (c).

The mean minimum and maximum temperatures in the glasshouse during pre-anthesis were 10.6 ± 0.4 °C and 20.8 ± 0.4 °C, respectively. During post-anthesis, mean minimum and maximum temperatures were 11.5 ± 0.3 °C and 25.6 ± 1.1 °C, respectively. Mean relative humidity was 66.7 ± 1.3% during pre-anthesis and 59.7 ± 1.1% during post-anthesis.
6.2.3 Measurements

When plants were at 50% anthesis and just before imposing the watering treatments, 3 separate set of pots from each genotype were randomly selected and number of tillers and emerged spikes recorded. Then the plants were harvested by cutting the shoots from the roots at the crown. Leaf area was measured using a portable leaf area meter (LI-3000, LI-COR Biosciences, Nebraska, USA) and shoot and leaves were dried in an oven at 60 °C for 48 hours and then weighed. Immediately after harvesting the shoots, the roots were recovered from the soil by repeated gentle washing and sieving on a 1.4-mm sieve to produce a clean sample, as described by Liao et al. (2006) and Palta et al. (2007). Roots were then dried in an oven at 60 °C for 72 hours and weighed.

Stomatal conductance ($g_s$) was measured on both sides of the flag leaf of the main stem using a Delta-T AP4 porometer (Delta-T Devices Ltd., Cambridge, UK) between 11:00 and 13:00 h on clear sunny days from anthesis and until the flag leaves in WS treatment dried and rolled completely. Leaf water potential ($\Psi_{leaf}$) was measured on clear sunny days at three different time points after withholding water, as reference of the development of the water deficits. Midday $\Psi_{leaf}$ was measured at (1) at anthesis, (2) when pot water content reached 60% of pot water holding capacity, just before watering was initiated through irrigation tubes to the bottom one-third of the soil column (167 °Cd for IGW-3262 and 99 °Cd for Drysdale), and (3) when changes in pot weight ceased in the WS treatments. $\Psi_{leaf}$ was measured using a Scholander-type pressure chamber (model 1000, PMS Instrument Co., Oregon, USA). The flag leaf was loosely covered with a plastic sheath before excision and during the measurement to avoid evaporation (Turner, 1988).

The amount of water applied to each pot each time was recorded. Pre-anthesis water use was calculated as the difference in weight of individual pot at planting and at anthesis plus the water applied in-between. As the soil surface was covered with beads from anthesis,
pre-anthesis water use represents evaporation from soil surface and transpiration by the plant. Post-anthesis water use was calculated as the difference in weight of individual pot at anthesis and at maturity plus the water applied in-between, which represents water transpired by the plant during that period. Post-anthesis water use efficiency (WUE$_{pa}$) was calculated as grain yield per unit water consumed after anthesis and total water use efficiency (WUE) was calculated as grain yield per unit total water consumed (pre and post anthesis water use). Final pot weight of the DD plants where the plants were permanently wilted ($w_f$) was recorded before harvest to calculate plant available water (PAW) as $(w_n - w_f)/(w_i - w_f)*100$, where $w_n$ is the weight of pot on the day of measurement and $w_i$ is the initial pot weight at saturation (Ritchie, 1981; Sadras and Milroy, 1996).

At final harvest, the number of spikes per plant and spikelets per spike were counted. Spikes were separated from shoots, oven dried at 40 °C for 6 to 7 days and hand threshed. The number and weight of grains per plant were also recorded. Harvest index (HI) was calculated as the ratio of grain yield to shoot biomass.

6.2.4 Statistical analysis

The data was analysed using the statistical software R 2.14.0 (R Development Core Team, 2011) and GraphPad Prism 6.03. Growth parameters at anthesis, before the initiation of drought treatments were analysed by performing unpaired t-test. Two-way ANOVA was performed with genotype and drought treatments as main effects to compare growth and yield parameters at final harvest. Multiple comparisons were done using the Least Significant Difference (LSD) test. Wherever an interaction effect was significant, results were interpreted based on the LSD value for interaction and main effects were not taken into account. As the genotypes attained anthesis stage on different days, time series measurements of $g_s$ and transpiration was plotted against thermal time calculated by accumulating daily mean temperature; anthesis being 0 °Cd of each genotype from which
watering was withheld from the treatments, WB and WS. Regression analysis of PAW against thermal time was carried out. Different models were compared based on extra sum of squares F test to select the best fit model.

6.3 Results

6.3.1 Phenology

The breeding line IGW-3262 reached anthesis 6 days (119 °Cd) earlier than the cultivar Drysdale (P < 0.01; Table 6.1). Hence terminal drought could not be induced in both genotypes at the same time. When watering was withheld completely from anthesis (WS), both IGW-3262 and Drysdale reached maturity 17 days (287 °Cd ) earlier than when they were well-watered (WW). When the bottom 30 cm of the pot was maintained well-watered (WB), Drysdale matured at the same time as it was in WW treatment, but IGW-3262 matured two days (42 °Cd) later than when it was in WW.

Table 6.1 Days to anthesis (n = 25) and physiological maturity (n = 4) when drought was induced from anthesis by withholding water completely (WS), withholding watering to 60% pot water capacity and then watering was restricted to the bottom 30 cm of the soil profile in the pot (WB) and when watering was maintained at 90% pot water capacity from anthesis to physiological maturity (WW). Data are means ± SEM. Means followed by different letters are significantly different between genotype (days to anthesis) or genotype and treatment (days to physiological maturity) at 95% level of significance.

<table>
<thead>
<tr>
<th></th>
<th>Days to anthesis</th>
<th>Days to physiological maturity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>WB</td>
</tr>
<tr>
<td>IGW-3262</td>
<td>77</td>
<td>151.5</td>
</tr>
<tr>
<td>Drysdale</td>
<td>83</td>
<td>149.3</td>
</tr>
</tbody>
</table>

6.3.2 Plant growth

At anthesis, just before withholding water to induce terminal drought, both genotypes had similar tiller number, leaf area, shoot and root biomass (P > 0.05 for each trait) (Figure 6.2).
Both genotypes had $3.6 \pm 0.7$ spikes emerged per plant ($P > 0.05$) and seminal roots had reached the bottom of the pot (Figure 6.3).

**Figure 6.2** Number of tillers (a), leaf area (b), shoot biomass (c) and root biomass (d) of the breeding line IGW-3262 and cultivar Drysdale at anthesis, just before induction of terminal drought. Error bars are SEM, $n = 3$.

**Figure 6.3** Soil column tipped outside the pot at anthesis to ensure that the roots had grown to the bottom of the pot by anthesis. The example shown is for IGW-3262
At final harvest, the number of tillers had significant genotype x treatment interaction effect (P = 0.02). Drysdale and IGW-3262 had the same number of tillers under well-watered conditions (Table 6.2) and was unaffected in WB. WS treatment reduced tiller number by 23% in Drysdale, but was not affected in IGW-3262.

Shoot biomass at final harvest had significant genotype x treatment interaction effect (P = 0.003). Under well-watered conditions, Drysdale had 16% more shoot biomass than IGW-3262, but Drysdale had 31% more shoot biomass than IGW-3262 in WB treatment (Table 6.2). Under WS conditions, both Drysdale and IGW-3262 had similar shoot biomass.

Root biomass at final harvest was similar in both genotypes (P = 0.5). WS treatment reduced root biomass by 29% in both genotypes (P = 0.0001; Table 6.2), but was not affected by WB treatment.

### Table 6.2 Number of tillers, shoot biomass and root biomass of IGW-3262 and Drysdale when drought was induced from anthesis by withholding water completely (WS), withholding watering to 60% pot water capacity and then watering was restricted to the bottom 30 cm of the soil profile in the pot (WB) and when watering was maintained at 90% pot water capacity from anthesis to physiological maturity (WW). Values are means (n = 4). Means within the same column followed by different letters are different at 95% level of significance. LSD values are at 95% level of significance.

<table>
<thead>
<tr>
<th></th>
<th>Tillers plant(^{-1})</th>
<th>Shoot biomass (g plant(^{-1}))</th>
<th>Root biomass (g plant(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IGW-3262</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>10.7 (^{a})</td>
<td>30.8 (^{b})</td>
<td>1.7 (^{a})</td>
</tr>
<tr>
<td>WB</td>
<td>9.2 (^{ab})</td>
<td>26.0 (^{c})</td>
<td>1.5 (^{a})</td>
</tr>
<tr>
<td>WS</td>
<td>10.7 (^{a})</td>
<td>14.2 (^{e})</td>
<td>1.2 (^{b})</td>
</tr>
<tr>
<td><strong>Drysdale</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>9.9 (^{ab})</td>
<td>35.7 (^{a})</td>
<td>1.7 (^{a})</td>
</tr>
<tr>
<td>WB</td>
<td>9.0 (^{bc})</td>
<td>34.0 (^{a})</td>
<td>1.7 (^{a})</td>
</tr>
<tr>
<td>WS</td>
<td>7.6 (^{c})</td>
<td>16.4 (^{d})</td>
<td>1.2 (^{b})</td>
</tr>
<tr>
<td>LSD (genotype x treatment)</td>
<td>1.46</td>
<td>2.11</td>
<td>NS</td>
</tr>
<tr>
<td>LSD (genotype)</td>
<td>0.83</td>
<td>1.21</td>
<td>NS</td>
</tr>
<tr>
<td>LSD (treatment)</td>
<td>1.02</td>
<td>1.49</td>
<td>0.20</td>
</tr>
</tbody>
</table>
6.3.3 Soil drying and leaf water potential

The soil drying rate was faster in Drysdale than in IGW-3262 when water was withheld completely from anthesis (P < 0.001; Figure 6.4). Drysdale reduced PAW to 10% within 130 °Cd after anthesis at a rate of 0.62 ± 0.04% °C⁻¹d⁻¹. Rate of decline was slower in IGW-3262 (0.39 ± 0.02% °C⁻¹d⁻¹) and PAW was reduced to 10% within 215 °Cd after withholding water at anthesis. Leaf water potential was maintained between –0.6 and –0.8 MPa in both genotypes when pots were well-watered (Figure 6.5). When water was withheld from anthesis, Ψ_leaf declined faster in Drysdale than in IGW-3262. In Drysdale, Ψ_leaf declined to –1.5 MPa in 99.5 °Cd, whereas IGW-3262 reached –1.5 MPa later by 167.7 °Cd. PAW corresponding to this Ψ_leaf was around 20% in both genotypes. When watering was restricted to the bottom 30 cm of the soil pot, Ψ_leaf in both genotypes recovered back to –0.7 MPa. When water was completely withheld from anthesis, Ψ_leaf fell to –2.7 MPa.

Figure 6.4 Soil water depletion of Drysdale and IGW-3262 with thermal time after anthesis when drought was induced by withholding water completely (WS). Pot soil water content is given as percentage of plant available water (PAW). Each point is the mean of four replicates. Error bars represents SEM.
Figure 6.5 Leaf water potential ($\Psi_{\text{leaf}}$) in Drysdale and IGW-3262 at three different times after anthesis when drought was induced from anthesis by withholding water completely (WS), withholding watering to 60% pot water capacity and then watering was restricted to the bottom 30 cm of the soil profile in the pot (WB) and when watering maintained pot water holding capacity at 90% from anthesis to physiological maturity (WW). Each value is the mean of four replicates. Error bars are SEM.

6.3.4 Stomatal regulation during post-anthesis

Under WB and WS treatments, $g_s$ declined in both genotypes as the soil water content lowered to 60% of pot water capacity (40% PAW) (Figure 6.6). The decline in $g_s$ was faster in IGW-3262 than in Drysdale. At 15 °Cd after watering was started to the bottom 30 cm of the soil profile, $g_s$ was 60% lower in IGW-3262 and 30% lower in Drysdale in the WB treatment than under well-watered conditions. $g_s$ recovered and was maintained similar to that under well-watered conditions within 110 °Cd after watering was started to the bottom 30 cm of the soil profile in both genotypes.
Chapter 6 – Root capacity to uptake water

Figure 6.6 Changes in stomatal conductance ($g_s$) (abaxial and adaxial leaf surfaces combined) with thermal time after anthesis in (a) IGW-3262 and (b) Drysdale when drought was induced from anthesis by withholding water completely (WS), withholding watering to 60% pot water capacity and then watering was restricted to the bottom 30 cm of the soil profile in the pot (WB) in relation to stomatal conductance when watering maintained pot water holding capacity at 90% from anthesis to physiological maturity (WW). Symbol ▲ represents the thermal time when watering to the bottom 30 cm of the soil profile was started in each genotype. Each value is the mean of four replicates. Error bars represent SEM.

Adaxial and abaxial $g_s$ fluctuated in IGW-3262 even under well-watered conditions, abaxial $g_s$ more than adaxial. Both $g_s$ declined in response to soil water deficit as the pot water capacity lowered to 60%. (Figure 6.7). After watering the bottom 30 cm of the pot, both adaxial and abaxial $g_s$ recovered slowly and were maintained similar to the WW plants (Figure 6.7). Under WB treatment, adaxial $g_s$ in Drysdale recovered similarly to the plants under WW treatment within 90 °Cd after watering the bottom 30 cm of the soil profile, whereas abaxial $g_s$ remained lower than WW plants, even after watering was supplied to the bottom 30 cm of the soil.
Figure 6.7 Adaxial (a,b) and abaxial (c,d) stomatal conductance ($g_s$) in IGW-3262 and Drysdale when drought was induced from anthesis by withholding water completely (WS), withholding watering to 60% pot water capacity and then watering was restricted to the bottom 30 cm of the soil profile (WB) and when watering maintained pot water holding capacity at 90% from anthesis to physiological maturity (WW). Each value is the mean of four replicates. Error bars are SEM.

6.3.5 Water use and water use efficiency

Under WW conditions, pre-anthesis water use in Drysdale (10.6 ± 0.19 L) was 12% higher than in IGW-3262 (9.3 ± 0.23 L; $P = 0.0003$), but post anthesis water use was similar in both genotypes (Table 6.3). There was a significant genotype x treatment interaction effect on post-anthesis water use ($P = 0.007$). Under WB treatment, water use was reduced by 30% in IGW-3262 and by 11% in Drysdale. Under WS condition, the water available during post anthesis was 22-23% of well-watered treatment in both genotypes.

Post-anthesis water use efficiency was higher in Drysdale than IGW-3262 (Table 6.3; $P > 0.0001$). $WUE_{pa}$ in both genotypes was 15-20% higher in WB and WS treatments ($P = 0.001$).
Total water use efficiency had significant genotype x treatment interaction effect (P < 0.0001). WUE was higher in Drysdale than IGW-3262 under all treatment conditions (Table 6.3). Under WB treatment, WUE was maintained similar to well-watered conditions in both genotypes, but was 50% lower in WS treatment.

Table 6.3: Post-anthesis water use and water use efficiency, and total water use efficiency (WUE) of IGW-3262 and Drysdale when water was withheld from anthesis by withholding water completely (WS), withholding watering to 60% of pot water capacity and then restricted watering to the bottom 30 cm of the soil profile in the pot (WB) and when watering maintained pot water holding capacity at 90% from anthesis to physiological maturity (WW). Post-anthesis WUE was calculated as grain yield per unit of post-anthesis water used (transpired) and total WUE was calculated as grain yield per unit of total water consumed. Each value is a mean of 4 replicates. Means within the same column with the same letters are not different at 95% level of significance.

<table>
<thead>
<tr>
<th></th>
<th>Post-anthesis water use (L plant(^{-1}))</th>
<th>Post-anthesis WUE (g grain L(^{-1}))</th>
<th>Total WUE (g grain L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IGW-3262</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>11.5(^{a})</td>
<td>1.12(^{e})</td>
<td>0.63(^{b})</td>
</tr>
<tr>
<td>WB</td>
<td>8.0(^{c})</td>
<td>1.29(^{d})</td>
<td>0.60(^{b})</td>
</tr>
<tr>
<td>WS</td>
<td>2.7(^{d})</td>
<td>1.38(^{c})</td>
<td>0.31(^{d})</td>
</tr>
<tr>
<td><strong>Drysdale</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>11.0(^{a})</td>
<td>1.45(^{b})</td>
<td>0.77(^{a})</td>
</tr>
<tr>
<td>WB</td>
<td>9.8(^{b})</td>
<td>1.74(^{a})</td>
<td>0.75(^{a})</td>
</tr>
<tr>
<td>WS</td>
<td>2.7(^{d})</td>
<td>1.82(^{a})</td>
<td>0.37(^{c})</td>
</tr>
<tr>
<td>LSD (genotype x treatment)</td>
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<td>NS</td>
<td>0.05</td>
</tr>
<tr>
<td>LSD (genotype)</td>
<td>0.6</td>
<td>0.14</td>
<td>0.03</td>
</tr>
<tr>
<td>LSD (treatment)</td>
<td>0.7</td>
<td>0.17</td>
<td>0.03</td>
</tr>
</tbody>
</table>

6.3.6 Yield and yield components

Grain yield had significant genotype x treatment interaction effect (P = 0.004). Drysdale had 24% higher grain yield than IGW-3262 under WW conditions (Table 6.4). Under WB conditions, Drysdale had similar grain yield to the WW treatment, while the grain yield of IGW-3262 was reduced by 23%. Under WS conditions, grain yield in both genotypes was reduced to less than 30% compared with the well-watered treatment.
The number of grains per plant was similar in both genotypes under WW conditions. When post-anthesis watering was limited to the bottom 30 cm of the pot, IGW-3262 reduced grain number by 30% and Drysdale by 16%. Under WS conditions, reduction in the number of grains was 56% and 43%, respectively in IGW-3262 and Drysdale.

There was a significant interaction effect of genotype x treatment for spikes per plant (P < 0.02). The number of spikes in IGW-3262 decreased by 25% and 61%, respectively in WB and WS treatments (Table 6.4). The number of spikes per plant was not reduced in Drysdale in WB treatment, but decreased by 42% in WS treatment. The number of spikelets per spike was 19.4 ± 0.3 in both genotypes (P = 0.06) and was not affected by the terminal drought treatments (P = 0.16).

The number of grains per spike varied between genotypes (P = 0.02), with Drysdale having 16% more grains per spike than IGW-3262. Post-anthesis watering treatments had no effect on number of grains per spike (P = 0.8; Table 6.4). 1000-grain weight under WW and WB treatments was 15-18% higher in Drysdale than IGW-3262 (P = 0.02). WS treatment reduced 1000-grain weight by 35% in IGW-3262 and 49% in Drysdale (P < 0.001). Harvest index was higher in Drysdale than IGW-3262 under all treatments (P < 0.001). Harvest index was reduced by 40% in WS treatment (P < 0.001), but was not affected in WB treatment.
Table 6.4 Yield and yield components of IGW-3262 and Drysdale when drought was induced from anthesis by withholding water completely (WS), withholding watering to 60% pot water capacity and then watering was restricted to the bottom 30 cm of the soil profile in the pot (WB) and when watering maintained pot water holding capacity at 90% from anthesis to physiological maturity (WW). Values are means (n = 4). Means within the same column with the same letters are not different at 95% level of significance. LSD values are at 95% level of significance.

<table>
<thead>
<tr>
<th></th>
<th>Grain yield (g plant⁻¹)</th>
<th>Grains per plant</th>
<th>Spikes per plant</th>
<th>Grains per spike</th>
<th>1000 grain weight (g)</th>
<th>Harvest Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IGW-3262</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>13.47       c</td>
<td>394.8 a</td>
<td>10.3 a</td>
<td>38.7 b</td>
<td>34.2 c</td>
<td>0.44 b</td>
</tr>
<tr>
<td>WB</td>
<td>10.34       d</td>
<td>275.6 c</td>
<td>7.7 b</td>
<td>36.9 b</td>
<td>37.7 bc</td>
<td>0.40 c</td>
</tr>
<tr>
<td>WS</td>
<td>3.71        e</td>
<td>172.3 d</td>
<td>4.0 d</td>
<td>38.5 b</td>
<td>23.9 d</td>
<td>0.26 d</td>
</tr>
<tr>
<td><strong>Drysdale</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>16.75       a</td>
<td>417.0 a</td>
<td>9.3 ab</td>
<td>45.0 a</td>
<td>40.2 ab</td>
<td>0.47 a</td>
</tr>
<tr>
<td>WB</td>
<td>15.05       b</td>
<td>349.4 b</td>
<td>8.2 b</td>
<td>43.0 a</td>
<td>43.2 a</td>
<td>0.46 a</td>
</tr>
<tr>
<td>WS</td>
<td>4.81        e</td>
<td>236.1 c</td>
<td>5.4 e</td>
<td>44.2 a</td>
<td>20.5 d</td>
<td>0.29 d</td>
</tr>
<tr>
<td>LSD (genotype x treatment)</td>
<td>1.39         NS</td>
<td>1.25</td>
<td>NS</td>
<td>4.62</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LSD (genotype)</td>
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<td>5.13</td>
<td>2.66</td>
<td>0.02</td>
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<tr>
<td>LSD (treatment)</td>
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<td>0.89</td>
<td>NS</td>
<td>3.26</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>
6.4 Discussion

The wheat cultivar Drysdale and the breeding line IGW-3262 differed in their post-anthesis water use when the soil dried from the top to the bottom but sufficient water was available in the bottom 30 cm of the soil profile in the 1 m pots. This difference is likely to be associated with differences in their root system capacity to capture water at depth (Passioura, 1983; Kirkegaard, 2007) and stomatal response to soil drying (Blum and Johnson, 1993). Post-anthesis water use in IGW-3262 was low when post-anthesis watering was limited to the bottom 30 cm of the soil profile in the pots (WB) while Drysdale maintained similar water use as in WW treatment. This indicates that the two genotypes differed in their ability to use the available soil water at depth, which was also observed in chapter 4. Lower root length and root biomass in the bottom segment of IGW-3262 compared with Drysdale as found in Chapter 4 indicates less exploration of soil volume and consequently less water uptake. IGW-3262 has thinner roots than Drysdale, as demonstrated by its high specific root length (SRL) (Saradadevi et al., 2014). Thinner roots may have lower root hydraulic conductivity in wheat (Zhao et al., 2005), depending on root anatomy and pathway of water flow (Bramley et al., 2009). Alternatively, Drysdale may be performing hydraulic lift that its shallow roots have access to water (Shen et al., 2011).

Difference in post anthesis water use was reflected in the grain yield of these two genotypes when watering was restricted to the bottom 30 cm of the soil profile. The grain yield in Drysdale in WB treatment was similar to when plants were well-watered. However, the grain yield of IGW-3262 was reduced by 23%, proportional to the reduction in post-anthesis water use. This is supported by Kirkegaard (2007) and Manschadi et al. (2006) that every extra millimetre of water captured during grain filling results in yield advantage.

Yield reduction in IGW-3262 when post-anthesis watering was limited to the bottom 30 cm of the soil profile in the pots (WB) resulted from a reduction in the number of spikes per
plant and consequently less grains per plant than when plants were well-watered (WW). This may be due to the reduction in g\textsubscript{s} observed in IGW-3262 soon after anthesis. Water deficit at anthesis is reported to affect the number of grains (Liu et al., 2005). However, 1000-grain weight was maintained similar to WW treatment. Gain filling was not affected as the g\textsubscript{s} in WB treatment returned to that of WW when watering was provided to bottom 30 cm of the soil profile. Reduction in the number of spikes to maintain 1000-grain weight has been reported in durum wheat under terminal drought conditions (Giunta et al., 1993).

Grain yield in Drysdale was higher than IGW-3262 when plants were well-watered (WW) throughout grain-filling, resulting in higher WUE. WUE in wheat is closely associated with pre-anthesis water use (French and Schultz, 1984). Drysdale had 18% higher pre-anthesis water use than IGW-3262, presumably as a consequence of difference in phenology. IGW-3262 reached anthesis 6 days earlier than Drysdale and it is possible that the early flowering had an effect on the growth and proliferation of the bottom roots (Palta and Gregory, 1997). Despite the differences in time to flowering between Drysdale and IGW-3262, physiological maturity occurred at a similar time in both genotypes. Thus, IGW-3262 had a longer grain-filling than Drysdale and hence expected to have a higher water use than Drysdale. However, under well-watered conditions, post-anthesis water use was similar in IGW-3262 and Drysdale, reflecting differences in their root capability to supply water to meet plant’s demand even under well-watered conditions.

The available soil water was exhausted faster by Drysdale than by IGW-3262 when watering was completely withheld from anthesis, which is supported by the observations reported in Chapter 4. Better water extraction efficiency of roots at depth might have allowed Drysdale plants to extract and use more water in transpiration, leading to rapid exhaustion of the available soil water (Sinclair et al., 1984). As a consequence of the fast soil water exhaustion, g\textsubscript{s} in Drysdale commenced declining at an earlier thermal time than
IGW-3262. This initial decline in $g_s$ when water was withheld from the soil profile could be in response to drying the top soil (Gollan et al., 1986; Blum and Johnson, 1993).

Faster soil drying rate in Drysdale than IGW-3262 suggests that Drysdale is less conservative in water use. Drysdale is a well-known drought tolerant cultivar, bred for high transpiration efficiency (Condon et al., 2004), but it has been classified as non-conservative in water use by Schoppach and Sadok (2012). This classification was mainly based on its transpiration response to VPD. The less sensitivity of Drysdale stomata to environmental factors observed in Chapter 4 is consistent with this conservative nature. In contrast, Drysdale was observed to close stomata in response to soil water deficit earlier than IGW-3262 in Chapters 4 and 5. Because of cloudy days, it was not possible to frequently measure $g_s$ after anthesis in this study. So it is unclear whether Drysdale or IGW-3262 closed stomata earlier with changes in soil water status. Regardless of that, the reduction in $g_s$ was greater in IGW-3262 than in Drysdale when watering was completely withheld from the pot. This is in contrast with the $g_s$ response of IGW-3262 in Chapters 3, 4 and 5 where $g_s$ reduction was greater in Drysdale. VPD in the glasshouse was higher during the initial soil drying phase and IGW-3262 stomata were much sensitive to environmental factors in chapter 4. Alternatively, intensity of water deficit experienced by IGW-3262 may be higher than Drysdale.

$\Psi_{leaf}$ indicates the intensity of water stress experienced by the plant. However, isohydric behaviour of IGW-3262 in contrast to anisohydric nature of Drysdale observed in Chapters 3 and 4 suggests that similar $\Psi_{leaf}$ in Drysdale and IGW-3262 under the WS treatment does not infer similar intensity of water stress. Remarkably lower $g_s$ in IGW-3262 during the time when the soil water was allowed to be depleted to reach 60% of pot water holding capacity suggests that stress level may be higher in IGW-3262 than Drysdale.
As soil dried from the top, it was assumed that by 60% pot water holding capacity, the top two-thirds of the soil pot would have completely exhausted the available soil water and the bottom one-third of the pot (30 cm) would be at its water holding capacity. If the roots of IGW-3262 were less active in extracting soil water from the bottom of the pot, it is likely that this genotype exhausted the soil water from the top of the pot. In contrast, roots of Drysdale may have extracted water more homogeneously throughout the soil profile. Detailed measurements of the development and intensity of the post-anthesis water deficits in different layers of soil profile were not made, but because of the differences between the two genotypes in their root system capacity to extract soil water from the bottom, it is possible that intensity of the water deficit in IGW-3262 may be higher than in Drysdale at similar pot weight.

Contrasting relative stomatal response of Drysdale and IGW-3262 in chapters 3, 4 and 5 and this experiment may also be related to root properties (Aston and Lawlor, 1979). The experimental procedures used in Chapter 4 resulted in higher soil compaction in the bottom segment and soil compaction not only affected root growth, but also its functionality (Dracup et al., 1992). The low capability of the bottom roots to extract water despite sufficient water was available, may be related to mechanical impedance and poor aeration (Tardieu et al., 1991; Atwell, 1993). Soil compaction was uniform throughout the soil profile in Chapters 3 and 5, but the pots were small and may have exhausted soil water very fast. In this study, soil compaction was conducive to root growth and therefore, root growth was profuse in the bottom one-third soil column at anthesis as evident in Figure 6.3. Despite profuse root growth in the bottom 30 cm of the soil profile, IGW-3262 was not able to maintain water use and grain yield in WB treatment similar to when the plants were well-watered. Not only root distribution in the lower soil profile is important, but also its hydraulic properties need to be favourable to allow extraction of water (Vadez, 2014). Hydraulic conductivity of wheat roots is variable (Bramley et al., 2007) and has been
shown to increase in response to transpiration demand in hydroponically grown plants (Vysotskaya et al., 2003; Vysotskaya et al., 2004). Drysdale roots may have superior hydraulic conductivity that aids water extraction in response to its high plant water demand maintained by its $g_c$. For instance, Drysdale roots have some compensatory adjustment to increase the uptake of water under water deficit condition by producing more thin roots (Saradadevi et al., 2014). Thinner new roots may imply more branching and hence more root tips, the region where water uptake is maximum in wheat (Bramley et al., 2009). Conversely, higher $\Psi_{\text{leaf}}$ observed in Drysdale may also result in higher transpiration pull facilitating water extraction from deeper soil profile (Aston and Lawlor, 1979; Turner and Begg, 1981). Consequently, higher water flux from the bottom roots in Drysdale than in IGW-3262 may have diluted the drying signals from the roots in the top dry soil sustaining stomatal conductance (Tardieu et al., 1992a).

Both adaxial and abaxial stomata in IGW-3262 responded to changing conditions similarly, but abaxial stomata seemed to be more sensitive in Drysdale. Increased sensitivity of abaxial stomata to water deficits and to exogenous ABA compared with adaxial stomata has been reported in several species like broad bean (Wang et al., 1998) and lupin (Henson et al., 1989b; Correia and Pereira, 1995). Less sensitivity of adaxial stomata in Drysdale to changes in soil water status and environmental factors is an important feature as this differential response may indicate minimal trade off between carbon assimilation and water loss. Drysdale clearly has a better ability to convert biomass into grain as indicated by a higher harvest index than IGW-3262 under all treatment conditions. However, root characteristics clearly show more prominence in regard to capture of available soil water effectively, even though there are chances that stomatal characteristics might have limited IGW-3262 root to extract water at their full capacity (Acuña and Wade, 2005).
6.5 Conclusions

Sustained post-anthesis water use is critical for grain yield maintenance in wheat under terminal drought. Grain yield differences in wheat genotypes Drysdale and IGW-3262 when top soil dried and soil water was available in the bottom 30 cm of the soil profile in the pots was related to their difference in post-anthesis water use, presumably as a consequence of their root capacity to extract water from deeper layers. Drysdale exhausted soil water faster than IGW-3262, sustained gs during minor water deficit confirming its non-conservative behaviour. Under conditions of sufficient water available at depth, the non-conservative approach post-anthesis appears to be more beneficial for yield. Under conditions of limited water availability, yield reduction was massive in both genotypes emphasising the importance of water available during grain filling for maintaining yield under terminal drought. Further investigation is needed to unravel the mechanisms that govern the observed difference in root capability to extract water between genotypes, probably involving root anatomical and hydraulic properties.
7 General discussion

7.1 Introduction

Wheat grown in the Mediterranean-type environment of south-western Australia is often exposed to end-of-season drought or terminal drought. This is because, in this environment, rainfall decreases and temperature and evaporative demand increase in the spring when wheat crops enter reproductive stage (Turner and Nicolas, 1987; Kobata et al., 1992). Post-anthesis water deficit affects grain yield (Turner and Nicolas, 1987; Kobata et al., 1992; Dias de Oliveira et al., 2013) due to poor grain filling resulting in shrivelled grains (Mitchell et al., 2013). Genotypes that maintain water uptake under terminal drought have high grain yield because this water is immediately used for grain filling (Passioura, 1983; Angus and van Herwaarden, 2001). Down-regulating stomatal conductance ($g_s$) under terminal drought could prevent rapid exhaustion of soil water and conserve soil water to facilitate water availability during grain filling. Stomatal closure in wheat under water deficit conditions is associated with the accumulation of the plant hormone, abscisic acid (ABA) in leaf tissues (Henson et al., 1989b; Ali et al., 1998). Studies in several species including wheat have been conducted to demonstrate the role of ABA in regulation of stomatal aperture, but not one has concentrated on its impact on water extraction and grain yield. To address this issue, four glasshouse experiments were conducted exposing different parts of the root system to dry soil after anthesis. The aim was to determine if root distribution in drying soil affects ABA accumulation and regulation of stomatal conductance to improve water use and grain yield under terminal drought.

7.2 Key research findings

Key findings of the experiments undertaken in this study and described in Chapters 3, 4, 5 and 6 of this thesis are summarised in Table 7.1.
Table 7.1 Summary of experiments described in this thesis including hypotheses tested, whether the hypotheses were accepted or rejected and key findings of each study.

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Hypothesis tested</th>
<th>Accepted or rejected</th>
<th>Key findings/ remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Genotypic variation in stomatal response to soil water deficit corresponds to increased accumulation of ABA in the leaves.</td>
<td>Partly accepted</td>
<td>Contrasting responses were found. Drysdale and IGW-3262 vary in their relationship between gs and leaf ABA.</td>
</tr>
<tr>
<td>4</td>
<td>More root density distributed in the drying upper soil layer is associated with early stomatal closure in response to soil water deficit.</td>
<td>Accepted</td>
<td>Drysdale had more root density in drying top soil and its stomata were more sensitive to soil water deficit than IGW-3262. Stomata started to close prior to marked increase in leaf ABA in both genotypes.</td>
</tr>
<tr>
<td>5</td>
<td>Xylem ABA is the major driver to initiate stomatal closure in Drysdale and IGW-3262. Stomatal sensitivity to xylem ABA concentration differs between Drysdale and IGW-3262.</td>
<td>Partly accepted</td>
<td>Factors other than xylem ABA concentration are also involved. It is not clear if stomatal sensitivity in these genotypes varies as IGW-3262 stomata had increased sensitivity to buffer solutions.</td>
</tr>
<tr>
<td></td>
<td>Stomatal sensitivity to xylem ABA concentration differs with pH of xylem sap.</td>
<td>Unable to test</td>
<td>IGW-3262 stomata are sensitive to pH, while Drysdale stomata are insensitive.</td>
</tr>
<tr>
<td>Chapter</td>
<td>Hypothesis tested</td>
<td>Accepted or rejected</td>
<td>Key findings/ remarks</td>
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<tr>
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</tr>
<tr>
<td>5</td>
<td>Stomatal sensitivity to xylem ABA concentration differs with water status of the plant.</td>
<td>Not proven</td>
<td>Higher concentration of ABA than normally found in water-stressed wheat plants was required to initiate stomatal closure, possibly due to high ( \Psi ) leaf after feeding.</td>
</tr>
<tr>
<td>6</td>
<td>Drysdale and IGW-3262 differ in their root capability to extract water from the bottom of the soil profile.</td>
<td>Accepted</td>
<td>Drysdale roots are more efficient at extracting water from deep in the soil profile than IGW-3262.</td>
</tr>
<tr>
<td>3,4,5,6</td>
<td>Early stomatal closure conserves water for use during grain filling which reduces the yield gap.</td>
<td>Partly accepted</td>
<td>Drysdale, which initiated stomatal closure earlier than IGW-3262 (Chapters 4 and 5) had better yield when top soil dried. However, it is not clear if early stomatal closure or its insensitivity to other environmental factors was advantageous for Drysdale. In addition, root properties also contributed to the yield benefit in Drysdale.</td>
</tr>
</tbody>
</table>
Contrasting responses in $g_s$ and leaf ABA concentration, as well as contrasting responses in $\Psi_{\text{leaf}}$ of four drought-tolerant genotypes were observed when one half (WD) or both halves of the split root system (DD) were exposed to drying soil (Chapter 3). Genotypes Drysdale and IGW-3262 had the most contrasting physiological responses; IGW-3262 reduced $g_s$ without any noticeable increase in leaf ABA concentration or drop in leaf water potential, but stomatal closure in Drysdale was accompanied by an increase in leaf ABA concentration and decline in leaf water potential. Both these genotypes had slightly different phenology, but the confounding effect of phenology on the difference in physiological response had been minimised by initiating drought treatment separately on each genotype when they reached anthesis. Hence, this contrasting physiological response of these two genotypes could be interpreted as different sensitivities of the root system and ABA production to soil drying. However, $g_s$ and $\Psi_{\text{leaf}}$ were only measured once after withholding water and no soil water measurement was possible due to the split pot design. Hence, it was not identified if Drysdale also started to close stomata before any noticeable change in $\Psi_{\text{leaf}}$ or leaf ABA or if stomatal sensitivity to soil water content differed among genotypes.

When the root systems of cultivar Drysdale and breeding line IGW-3262 were horizontally split and either the top half of the total root system (DW) or the whole root system (DD) was exposed to terminal drought (Chapter 4), Drysdale initiated stomatal closure earlier than IGW-3262. Drysdale had more transpiring area and root biomass in the upper 0–35 cm of the soil profile than IGW-3262 resulting in slightly earlier and faster soil water depletion. The soil water content corresponding to the thermal time at which Drysdale started reducing $g_s$ was higher than that at which IGW-3262 started closing its stomata. The $g_s$ reduction in Drysdale at 50% pot soil water content while $g_s$ in IGW-3262 was maintained similar to well-watered plants (Chapter 5) confirms that Drysdale stomata are
more sensitive to soil water deficit than IGW-3262. Considerably higher root biomass distributed in the drying top segment in Drysdale (Chapter 4) may have served as a large source for signal generation resulting in relatively higher leaf ABA concentration and subsequent stomatal closure (Martin-Vertedor and Dodd, 2011).

Stomatal conductance also varied in IGW-3262, even under well-watered conditions, suggesting a possible stomatal response to environmental conditions such as temperature, humidity, CO₂ concentration, radiation or VPD (Raschke, 1975; Xue et al., 2004). Climatic conditions were not constant in the glasshouse across measurement days as indicated by the fluctuations in VPD. However, it is not clear if the gs response matches the leaf-to-air VPD, the important source of variation of gs (Bunce, 1996), which was not measured in this study. In addition to environmental factors, other factors such as soil compaction (Tardieu et al., 1991) and nitrogen uptake (Wilkinson et al., 2007) may also have affected gs. On the other hand, Drysdale stomata appeared to be less sensitive to external factors as indicated by its relatively stable gs. It is not clear if ABA is involved in the stomatal closure affected by these factors other than soil moisture deficit or how the interaction between soil water deficit and external factors affects the ABA concentration and gs. Analysis of ABA metabolites suggest possible difference in ABA metabolism between Drysdale and IGW-3262, which may influence the observed differential sensitivity of stomata in these genotypes to soil water deficit and other unknown factors and their contrasting gs–leaf ABA relationship.

When the top soil dried after anthesis, initiation of stomatal closure preceded any remarkable increase in leaf ABA, suggesting that signals transmitted through xylem may be the driver for the initial gs response to water deficit in both genotypes (Zhang and Davies, 1990a; Tardieu et al., 1992b). Thus, it appears that stomatal responses in wheat under
terminal drought occur in two stages: primarily in response to drying signals from the root and secondarily in response to a leaf-derived signal (Xiong et al., 2006; Du et al., 2013).

It is not clear what served as the primary signal to initiate stomatal closure as no increase in root ABA was observed under terminal drought treatments (DW and DD, Chapter 4). However, feeding exogenous ABA to detached wheat leaves could initiate stomatal closure similar to that of intact plants under drought only at high concentrations than that reported in the xylem sap of water-stressed wheat plants. This indicates that xylem ABA may not be the sole driver of stomatal regulation in wheat. Considering the strong relationship between $g_s$ and leaf ABA in Drysdale, it seems that leaf ABA also plays an important role in stomatal regulation in wheat. However, genotypic variation may exist in this relationship as suggested by weak relationship in IGW-3262, which may be associated with possible stomatal sensitivity differences between these genotypes. For example, difference in uptake of $\text{NO}_3$ from the soil as the water uptake slows down due to soil drying can result in alkalinisation of xylem and subsequent increased stomatal sensitivity to ABA (Wilkinson et al., 2007).

To test if there are differences between Drysdale and IGW-3262 in stomatal sensitivity to xylem ABA concentration and pH, flag leaves from well-watered plants were fed with buffer solutions of different concentrations of ABA at pH 6 and 7. Stomatal closure in IGW-3262 in response to 0 ABA buffer solutions was unexpected and confirmed its sensitivity to factors other than ABA, possibly osmolality of the solution (Kelly et al., 2013). Alternatively, lack of signals from the roots may have restricted stomatal opening in IGW-3262. For example, continuous supply of cytokinin from the roots was essential to keep the stomata open in maize (Blackman and Davies, 1985). IGW-3262 stomata were also sensitive to the solution pH indicating its sensitivity to a multitude of factors other than
ABA. Thus, increased sensitivity of IGW-3262 stomata to factors other than ABA explains the weak relationship between $g_s$ and leaf ABA in IGW-3262.

The observed differences in stomatal properties between Drysdale and IGW-3262 did not result in any yield benefit under conditions of limited post-anthesis water availability as when watering was withheld completely after anthesis (Chapter 4). However, under conditions of available water at depth, yield benefit was observed in Drysdale, which initiated stomatal closure earlier in response to soil water deficit (Chapters 4 and 5), but demonstrated less sensitivity to other possible variable environmental conditions. Drysdale had 100% higher grain yield than IGW-3262 when watering was withheld from the top 0–30 cm of the soil profile. Nevertheless, compared with well-watered treatments, Drysdale also reduced yield by 50% when water was withheld from the top segment of the pot, similar to the yield reduction observed in field-grown wheat under post-anthesis water stress (Dias de Oliveira et al., 2013). The yield reduction in these genotypes, even when sufficient water was available in the bottom segment for plant extraction, suggests root limitation to extract water at depth. The soil in the bottom segment was highly compacted which probably affected not only root growth, but also root functionality (Tardieu et al., 1992a). However, Drysdale clearly fills grain faster and is better able to translocate biomass from shoots to grain, probably due to its better root efficiency to extract water from deeper soil profile.

In a 1-m soil profile with water available in the bottom 30 cm of the soil profile while the top soil dried from anthesis, no significant yield reduction was observed in Drysdale. IGW-3262, on the other hand, reduced yield by 25%. Yield reduction in IGW-3262 was proportional to the reduction in post-anthesis water use. The failure of IGW-3262 bottom roots to extract water from depth may be due to its reduced hydraulic properties which may be linked either to root anatomy or transpiration pull driven by stomatal regulation.
Chapter 7 – General discussion

Root anatomy and hydraulic properties of these genotypes were not studied in this thesis. However, the relatively lower SRL in Drysdale (Chapter 3 and 4) implies thicker roots than in IGW-3262. Thicker roots should have lower hydraulic resistance to water flow (Zhao et al., 2005). However, this varies with root anatomy and the preferred pathway of water flow (Bramley et al., 2009). Alternatively, wheat roots have the capacity to alter hydraulic resistance (Bramley et al., 2007), and increased root hydraulic conductivity (inverse of hydraulic resistivity) to meet the transpiration demand is mediated by ABA (Vysotskaya et al., 2003; Vysotskaya et al., 2004). Drysdale roots may have increased the hydraulic conductivity of bottom roots when the top soil was completely exhausted of water. Moreover, Drysdale produced more thin roots in the wet compartment when the other half of the pot was dried (Chapter 3). More root production in the wet region of the soil profile indicates better water extraction as wheat roots absorb water predominantly through the region near the root tip (Bramley et al., 2009). Further research should examine root hydraulic properties and the role of ABA in modifying both root and stomatal conductance to balance water extraction and transpiration.

7.3 Future directions and implications

Terminal drought is the most detrimental abiotic stress affecting wheat grain yield in Mediterranean-region of south-western Australia. With the changing climatic conditions that forecasts more frequent exposure to terminal drought in this region, identifying physiological traits that reduce yield gap is essential. This study identified that the key to sustaining grain yield under terminal drought conditions is a combination of gs and root properties to facilitate better post-anthesis water use. Although we do not yet fully understand how gs is linked to root properties in wheat, this thesis provides the foundation for further research. Root properties that need further investigation include root morphology, architecture and hydraulic properties in a wide range of wheat germplasm.
Establishing the link between $g_s$ and root hydraulic conductance may identify potential physiological traits that plant breeders can select for to enhance grain yield under terminal drought conditions.

This study identified contrasting stomatal sensitivity to soil water deficit in drought tolerant wheat genotypes. While Drysdale stomata were more sensitive to soil water deficit, IGW-3262 stomata appear to respond to factors other than soil water deficit. This study did not confirm the factors to which IGW-3262 stomata were sensitive, but suggests it may be related to environmental factors. Under terminal drought, where every extra millimetre of water adds to grain yield, less stomatal sensitivity to changing atmospheric conditions as observed in Drysdale, would be beneficial when water is available in the soil. Sensitive stomata to atmospheric variation would limit water uptake and grain yield even though sufficient water is available in the soil for extraction. For example, a very short period of reduced $g_s$ at anthesis (chapter 6) reduced the number of grains considerably in IGW-3262. However, once the soil water level starts declining, sensitive stomata to water deficit conditions would extend the grain filling period by slowing down the water use. Further investigations should be carried out under both controlled and field conditions to demonstrate how the interaction of environment and soil water deficit affects $g_s$.

This study further identifies that the genotypes differ not only in their $g_s$ response to drying soil and other unknown factors, but also in its relationship with ABA accumulation in their leaves. IGW-3262 stomata, which were sensitive to factors other than soil water deficit was also sensitive to non-ABA factors. Drysdale and IGW-3262 possibly differ in ABA metabolism, which could influence stomatal sensitivity to soil water deficit and atmospheric conditions. In addition to $g_s$, ABA is reported to regulate root hydraulic conductance in wheat (Vysotskaya et al., 2003; Vysotskaya et al., 2004). Hence, it is possible that ABA is the linking factor modulating $g_s$ and root hydraulic conductance to
regulate water use. If it is possible to obtain xylem sap from mature wheat plants under water deficit conditions the mechanism behind ABA-mediated stomatal regulation and water use could be further unravelled. Future studies involving more wheat genotypes are required to establish genotypic variation in gs response to water stress focussing on ABA. Accumulation of ABA in the leaf tissue under drought could probably be the trait to select for. Then, it would be beneficial to screen larger number of genotypes as collecting leaf samples is easier and convenient than measuring gs in the field. This is important for developing improved wheat cultivars capable of maintaining yield under terminal drought.
References


Dodd, I. C., Egea, G. & Davies, W. J. 2008b. Accounting for sap flow from different parts of the root system improves the prediction of xylem ABA concentration in plants grown with heterogeneous soil moisture. *Journal of Experimental Botany*, 59, 4083-4093.


exchange parameters and ribulose 1,5-bisphosphate carboxylase activation in
wheat. Environmental and Experimental Botany, 32, 403-410.


Hose, E., Steudle, E. & Hartung, W. 2000. Abscisic acid and hydraulic conductivity of

Physiology, 45, 529-530.

Hurd, E. A. 1968. Growth of roots of seven varieties of spring wheat at high and low
moisture levels. Agronomy Journal, 60, 201-205.

drought-induced abscisic acid accumulation on the yield and water use of spring
wheat. The Journal of Agricultural Science, 102, 341-351.

their seminal roots only. Effects on plant development, xylem transport, mineral
nutrition and the flow and distribution of abscisic acid (ABA) as a possible shoot to

Ji, X., Dong, B., Shiran, B., Talbot, M. J., Edlington, J. E., Hughes, T., White, R. G.,
Gubler, F. & Dolferus, R. 2011. Control of abscisic acid catabolism and abscisic
acid homeostasis is important for reproductive stage stress tolerance in cereals.
Plant Physiology, 156, 647-662.

ABA flux and concentration in leaves of maize and Commelina communis. Journal
of Experimental Botany, 47, 1085-1091.

Experimental Botany, 49, 387-398.

Kelly, G., Moshelion, M., David-Schwartz, R., Halperin, O., Wallach, R., Attia, Z.,
Belausov, E. & Granot, D. 2013. Hexokinase mediates stomatal closure. The Plant
Journal, 75, 977-88.


use on wheat yield. Australian Journal of Agricultural Research, 58, 303-315.

deficits and grain filling of spring wheat. Crop Science, 32, 1238-1242.

Turner, N. C. (eds.) Adaptation of plants to water and high temperature stress. New
York: John Wiley & Sons.


Richards, R. A. & Passioura, J. B. 1989. A breeding program to reduce the diameter of the major xylem vessel in the seminal roots of wheat and its effect on grain yield in rain-fed environments. *Australian Journal of Agricultural Research*, 40, 943-950.


Contrasting stomatal regulation and leaf ABA concentrations in wheat genotypes when split root systems were exposed to terminal drought

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A B S T R A C T

Wheat grown in the Mediterranean-type environments of southern Australia is often exposed to end-of-season drought (terminal drought). During the development of terminal drought, soil dries from the topsoil to the profile exposing the upper part of the root system to water stress while deeper roots may still be able to access deeper soil water for grain filling. It is hypothesised that the part of the root system exposed to drying soil signals abscisic acid (ABA) production and the corresponding rise in ABA concentration in leaves causes partial stomatal closure, regulating the extraction of available water at depth. In the first step to test this hypothesis, a greenhouse experiment was conducted to identify contrasting stomatal response to terminal drought and production of ABA using four wheat genotypes adapted to different soil moisture environments. Terminal drought was induced by withholding water from anthesis in one-half (WD) or both halves (DD) of the root system using split pots. Stomatal conductance decreased in all four genotypes in WD plants, but leaf ABA concentration and leaf water status differed. The cultivar Drysdale had higher leaf ABA concentrations and lower stomatal conductance, but leaf water status decreased. Leaf ABA concentration did not increase in WD plants of the breeding line GWS-3252, but stomatal conductance decreased and leaf water status was unchanged. All the genotypes behaved similarly under DD conditions, with increased leaf ABA concentration, lower stomatal conductance and severely dehydrated leaves. The possible causes of the above differences in leaf ABA concentration despite similar stomatal behaviour in wheat genotypes are discussed.

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1. Introduction

Wheat is one of the world’s staple food crops and is Australia’s most important grain crop (ABS, 2012). Terminal drought which occurs during post-anthesis (Kobata et al., 1992; Loss and Siddique, 1994) significantly reduces wheat grain yield (Saini and Aspinall, 1981; Simane et al., 1993). Due to decreasing rainfall and rising temperatures (Turner and Asseng, 2005), the frequency of exposure to terminal drought is predicted to increase for dryland wheat growing regions (Farre and Foster, 2010). Terminal drought induces grain abortion (Rajala et al., 2009) and affects grain filling (Rajala et al., 2009; Saini and Aspinall, 1981), resulting in shrivelled grains (Mitchell et al., 2013) and thus, reducing both grain yield (Dias de Oliveira et al., 2013) and quality (Gooding and Davies, 1997). Water used during post-anthesis plays an important role in determining wheat yield (Manzuddi et al., 2006; Passioura, 1993; Siddique et al., 1999). Hence, maintaining of water uptake during grain filling is critical (Richards and Passioura, 1989) under terminal drought, which can be achieved by minimising water loss through regulation of stomatal conductance.

Stomatal regulation in response to soil dryness is affected by the root’s ability to sense the drying of surrounding soils and communicate this information to the shoot (Gallai et al., 1985; Passioura, 1988b). Abscisic acid (ABA) is strongly advocated as the major signal involved in this root to shoot communication process (Dodd, 2005; Zhang and Davies, 1987), despite evidence that other chemicals are also involved (Munn and King, 1988; Yan et al., 2013) and/or signals (Davies and Zhang, 1991).
ABA is considered mainly as a root-derived hormone regulating stomatal closure in response to water stress (Hartung et al., 2002; Sivak et al., 1995). However, a lack of correlation between xylem ABA and stomatal conductance (Ali et al., 1998; Atkinson et al., 1989), but a better correlation with leaf ABA (Henson et al., 1989) indicates that stomatal regulation in response to soil dryness is related to the accumulation of ABA in leaf tissues (Mahdoll et al., 2011), at least in wheat.

Wheat genotypes differ in their ability to produce ABA (Quattre, 1980; Quatre and Henson, 1981) and in regulating stomata (Blum and Johnson, 1993) when exposed to drying soil and hence, may differ in their ability to save water. As the soil starts drying, the top part of the soil profile begins to dry while soil moisture is still available at depth. Thus, the part of the root system in the top soil profile will experience low soil water potential (Passioura, 1988a) while the deeper part of the root system has access to soil moisture (Chang and Davies, 1990). Therefore, although water may still be available at depth, roots in the top part of the soil profile may produce a signal that induces ABA production and the subsequent increase in ABA concentration in leaves may cause partial stomatal closure and water conservation. This behaviour has been observed in some horticultural crops and where partial root zone or deficit irrigation is used (Sobell et al., 2004; Stoll et al., 2000), but it is not known if this occurs in wheat. Wheat genotypes may differ in root system characteristics that detect and signal drying soil conditions, particularly since wheat root systems are comprised of two types of roots and there is genotypic variation in root system architecture (Manschadi et al., 2008; Fakta and Watt, 2009; Fakta et al., 2011). This paper presents results from an experiment conducted as part of a larger study examining mechanisms of water conservation involving stomatal regulation and ABA production by roots in response to soil drying in wheat. To mimic partial root zone drying, a split-root system was used to expose half of the root system to terminal drought-drying soil from anthesis. The dry half of the root system simulates roots exposed to low soil water potential (shallow roots) while the wet half simulates roots exposed to high soil water potential (deeper roots under field conditions). This approach was more feasible for an initial screening study to identify contrasting responses in stomatal regulation and ABA production to terminal drought rather than the large, deep pots that would be required to simulate terminal drought in the vertical soil profile. Four genotypes were selected for comparison based on their adaptation to contrasting environments in soil moisture (two commercial cultivars) or with putative drought tolerance based on yield performance across a range of dryland environments (two advanced breeding lines). We expected genotypic differences in stomatal closure corresponding to difference in ABA concentration in leaves in response to soil drying around half of the root system.

2. Materials and methods

2.1. Plant material

Four wheat (Triticum aestivum L.) genotypes, including two commercial cultivars (Drysdale and Wyalkatchem) and two advanced breeding lines (IGW-3119 and IGW-3262), putatively adapted to drought were grown in specially-designed split pots. The cultivar Drysdale, commercially released in 2001, is one of the most common cultivars currently grown in Western Australia and is adapted to low rainfall regions (Penny, 2002). The wheat-belt in south-west Western Australia has predominately intermittent rainfall and sandy-loam soils. The cultivar Drysdale, commercially released in 2002, has high leaf level transpiration efficiency based on its low discrimination against the carbon isotope $^{13}C$ compared with $^{13}C$ (Condron et al., 2004). This cultivar was designed for environments with predominately stored soil moisture. The advanced breeding lines IGW-3119 and IGW-3262, provided by Dr. Dan Mullen from InterGrain Pty Ltd. are putatively adaptable to dryland environments (D. Mullen, InterGrain, pers. comm.).

2.2. Split pot and growing conditions

Split pots were designed to split the root system in two halves and maintain one half in moist soil while allowing the other half to dry. The split pots were constructed from 30 cm long and 15 cm diameter polyvinyl chloride columns, cut vertically into two equal halves. A vertical plastic partition wall was placed between the two halves before rejoining using waterproof tape (Fig. 1). The width of the vertical wall was 2 mm greater than the outer diameter of the pot and its bottom was so aligned that it was 1 mm greater than the bottom of the pot to keep the two compartments hydraulically isolated. The whole assembly was held tight using cable ties.

Both halves of each pot were filled with a uniform soil mixture to a depth of 26 cm. The soil was a reddish-brown sandy clay loam obtained from a field site at Bindi Bindi in the South Australian grainbelt, mixed with 40% coarse river sand to improve drainage. The soil–sand mixture (approximately 9 kg per pot) had a pH of 7.8, electrical conductivity of 12.65 mS m$^{-1}$ and water holding capacity of 0.231 kg kg$^{-1}$ . Nutrients were supplied through slow release fertiliser pellets (Osmocote®; N-P-K 13:13:4.9) mixed into the top 0.1 m soil before planting at the rate of 10 g per pot and weekly application of water soluble fertiliser (Haifa Poly-feed; N-P-K 19:8:4.15:8 with magnesium and micronutrients) was applied until anthesis. Uniform sized seeds were germinated in Petri dishes on moist filter paper at room temperature for three days. Germinated seeds were transferred individually to a 2.0 cm long flexible tube, which was fixed in a groove on the top edge of the partition between the pot compartments (Fig. 1). The seminal roots were carefully diverted into the two compartments. In order to avoid dehydration of roots due to exposure, the base of the plant was covered with aluminium foil until the plant established. Every 2–3 days, emerging new roots were carefully diverted to both compartments, to ensure that root growth was equally distributed in both compartments. The four genotypes were grown in an evaporative-cooled glasshouse at The University of Western Australia, Perth, WA [31° 91’S, 115° 83’E] from June to October 2011. The mean minimum and maximum temperatures in the glasshouse during the experiment were 12 ± 2 and 26 ± 2°C respectively and mean relative humidity was 52 ± 6%. The four genotypes were grown on five benches in a completely randomised block design with five replicates. Pots were rotated weekly to minimise spatial variability. The pots were watered on alternate days close to pot water holding capacity until anthesis—285, Zadoks’ growth scale for cereals (Zadoks et al., 1974) when the wet and dry treatments were applied. Anthesis for each genotype was determined when anthers had emerged from the main stem of 50% of plants.

2.3. Treatments

At anthesis, pots from each genotype were divided into three groups of 10 pots. In the first group, both compartments of each pot were well-watered (WW). In the second group, water was withheld from one compartment in each pot (WD), and in the third group, water was withheld from both compartments of each pot (DD). Wet compartments were watered by hand everyday to maintain them close to soil water holding capacity. To prevent loss of water from the soil through evaporation, the soil surface in each pot compartment was covered with a 3 cm layer of white plastic beads.
2.4. Measurements

Shoot and root biomass was measured in all four genotypes three times during the experiment: (1) at anthesis, before terminal drought was induced; (2) when the plants in the DD treatment showed permanent wilting symptoms, which was at 180 days of treatment; and (3) at the final harvest. Before each sampling, the number of tillers per plant was recorded; the shoots were then cut from the roots at the crown. Leaf area was measured at anthesis and at the second harvest. Measurements were made using a portable leaf area metre (LI-3000, LI-COR Biosciences, Nebraska, USA). Shoot and leaves were separately dried in an oven at 60 °C for 48 h and then weighed. Immediately after harvesting the shoots, each split pot was opened and the roots in each half were recovered from the soil by repeated gentle washing and sieving on a 1.4 mm sieve to produce a clean sample, as described by Liao et al. (2000) and Patta et al. (2007). Roots were then dried in an oven at 60 °C for 72 h and weighed. At the second harvest, root length as well as root dry weight was measured after the roots were recovered from the soil in each half. Roots were stained for 30 min with 0.1% (w/v) methylene blue, placed in about 3 mm of water in a glass tray (0.2 x 0.3 m), and untangled with a plastic spatula to minimise overlapping. The glass tray was placed on a flatbed scanner with transparency unit (Epson STD4800). Scanning was done at 400 dpi resolution and the images were analyzed for total root length using WinRHIZO Pro 2008b (Regent Instruments Inc., Canada). Root material was then dried and weighed. Specific root length (SRL) was calculated as root length per unit biomass of root.

At final harvest, the number of spikes per plant and spikelets per spike were counted. Spikes were separated from shoots, oven dried at 40 °C for 6–7 days and threshed by hand. The number and weight of grains per plant were also recorded. Harvest index (HI) was calculated as the ratio of grain yield to shoot biomass.

Leaf water potential (ΨLwf), stomatal conductance (gs), transpiration and leaf net photosynthesis rate were measured on the flag leaf of the main stem of five replicate plants just before the second harvest, which was between 81 and 94 DAS (permanent wilting in DD treatment). Measurements were made between 10.30 am and 1.30 pm, on days with clear sky. Rates of leaf net photosynthesis, gs and transpiration were measured with a Li-COR gas-exchange system (Li-6400, LI-COR Biosciences, Nebraska, USA) with LED light source on the leaf chamber. In the Li-COR cuvette, CO2 concentration was set to 380 μmol mol⁻¹ and LED light intensity 950 μmol m⁻² s⁻¹, which is the average saturation intensity for photosynthesis in wheat (Austen, 1990). Immediately after these measurements were made, ΨLwf was measured using a Scholander pressure chamber (model 1000, PMS Instrument Co., Oregon, USA). The flag leaf was loosely covered with a plastic sheet before excision and during the measurement to avoid evaporation (Turner, 1988).

2.5. ABA sampling and analysis

After gas exchange and ΨLwf measurements, the same flag leaf was snap frozen in liquid nitrogen and stored at −80 °C. ABA analysis was done on two genotypes contrasting in stomatal conductance in response to soil drying. Analysis was carried out as per the protocol described by Speirs et al. (2013). Briefly, 50–100 mg of ground frozen tissue was extracted overnight at 4 °C in 500 μL of 20% aqueous methanol. After centrifugation, a deuterated internal standard (400 μL, containing D₃-7,7,7-PA and -dihydroxyphenic acid, D₅-4,5,8,8,8-ABA-GE and D₅-3,5,5,5,7,7,7-ABA, all at a concentration of 10 ng·mL⁻¹) was added to the supernatants. Phenomenex SPI columns (50 mg·mL⁻¹, 88-S1100-AW-E) were equilibrated with 1 mL methanol and 1 mL nanopure water, as per the manufacturer’s directions. The samples were loaded onto the columns, washed with 20% aqueous methanol (1 mL), and eluted with 90% aqueous methanol (1 mL). An aliquot (50 μL) of the eluate was then dried in a vacuum centrifuge for preparation in analysis by liquid chromatography/mass spectrometry (LC-MS/MS). The dried leaf extracts were dissolved in 50 μL aqueous acetonitrile (10% with 0.05% acetic acid) and 20 μL was analysed by LC-MS/MS (Agilent 6410). Separations were carried out on a Phenomenex C18(2) 75 mm x 4.5 mm x 5 μm columns at 40 °C. Solvents were nanopure water and acetonitrile, both with 0.05% acetic acid. Samples were eluted with a linear 15 min gradient starting at 10% acetonitrile and ending at 90% acetonitrile. Compounds were identified by retention times and multiple reaction monitoring.
2.6. Statistical analysis

Two-way ANOVA was conducted using the statistical software R 2.14.0 (R Development Core Team, 2011). To meet the assumptions of ANOVA, normal distribution and equal variance, data were subjected to square root transformation before analysis where necessary. To check if withholding water from one half of the pot affected the distribution of roots between wet and dry compartments, a two-way ANOVA was conducted with genotype and drought treatments (wet and dry) of each compartment as dependent variables. Multiple comparisons were done using the least significant difference (LSD) test using the R package agricolae (Mendiburu, 2010). Whenever an interaction effect was significant, results were interpreted based on the LSD value for interaction and main effects were not taken into account.

3. Results

3.1. Phenology

All genotypes except the line IGW-3262 reached anthesis at 74 to 75 DAS. IGW-3262 reached anthesis at 71 DAS. When watering was continued in both halves of the split root system until maturity (WW), IGW-3262 was the first to attain physiological maturity [when 90% of the flag leaf turned yellow and glumes lost green colour in 50% of the plants for each genotype] and Wyalkatchem the last (Table 1). When water was withheld from one half of the split root system (WD), all genotypes except IGW-3262 reached maturity 9–12 days earlier than those plants in which both halves of the root system were well-watered (WW). IGW-3262 plants were similar under both WD and WW treatments. When water was withheld from both halves of the split root system (DD), genotypes attained physiological maturity 16–24 days earlier than under the WW treatment (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Anthesis (DAS)</th>
<th>Physiological maturity (DAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>WD</td>
</tr>
<tr>
<td>IGW-3119</td>
<td>75</td>
<td>139</td>
</tr>
<tr>
<td>Drysdale</td>
<td>75</td>
<td>137</td>
</tr>
<tr>
<td>Wyalkatchem</td>
<td>74</td>
<td>140</td>
</tr>
<tr>
<td>IGW-3262</td>
<td>71</td>
<td>131</td>
</tr>
</tbody>
</table>
Table 2
Leaf water potential (Ψ<sub>leaf</sub>), stomatal conductance (g<sub>s</sub>), leaf transpiration rate and net photosynthetic rate of four wheat genotypes at second harvest (179-190 Cd after anthesis) that were well-watered (WW) or watered withholding from one (WD) or both (DD) halves of the split root system from anthesis. Values are the means of five replicates. LSD values are at 0.05 level of significance.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>WW</th>
<th>WD</th>
<th>DD</th>
<th>WW</th>
<th>WD</th>
<th>DD</th>
<th>WW</th>
<th>WD</th>
<th>DD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICGW-3119</td>
<td>-1.42</td>
<td>-1.68</td>
<td>-3.32</td>
<td>386</td>
<td>281</td>
<td>40</td>
<td>7.25</td>
<td>1.94</td>
<td>0.16</td>
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<tr>
<td>Drysdale</td>
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<td>-1.74</td>
<td>-3.71</td>
<td>376</td>
<td>245</td>
<td>40</td>
<td>7.24</td>
<td>1.53</td>
<td>0.13</td>
</tr>
<tr>
<td>Wyalkatchem</td>
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<td>-3.98</td>
<td>358</td>
<td>234</td>
<td>40</td>
<td>7.82</td>
<td>1.69</td>
<td>0.18</td>
</tr>
<tr>
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<td>-2.80</td>
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<td>125</td>
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</tr>
<tr>
<td>LSD (treatment)</td>
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<td></td>
<td></td>
<td>NS</td>
<td></td>
<td>1.3</td>
</tr>
<tr>
<td>LSD (genotype x treatment)</td>
<td>0.43</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
<td>2.5</td>
</tr>
</tbody>
</table>

3.2. Ψ<sub>leaf</sub> gas exchange and leaf tissue ABA

There was a significant interaction of genotype x treatment for Ψ<sub>leaf</sub> (P=0.002). Ψ<sub>leaf</sub> did not differ between genotypes under well-watered conditions (WW) (mean=1.3 ± 0.05 MPa; Table 2). When watering was withheld from one half of the root system (WD) from anthesis, Ψ<sub>leaf</sub> decreased to -1.8 MPa in Drysdale and Wyalkatchem, but did not decrease in ICGW-3262 and remained around -1.3 MPa, similar to WW plants. Withholding water from both halves of the root system (DD) reduced Ψ<sub>leaf</sub> in Drysdale and ICGW-3262 to -2.8 MPa, while in ICGW-3262 and Wyalkatchem, Ψ<sub>leaf</sub> decreased to -3.3 and -3.8 MPa, respectively.

Main effects of genotype and treatment were significant for stomatal conductance (P=0.002 and P=0.001, respectively), but the interaction was not significant (P=0.7). Stomatal conductance (g<sub>s</sub>) in all genotypes was similar under well-watered conditions with a mean value of 377 mmol m<sup>-2</sup> s<sup>-1</sup> (Table 2). Withholding water from one (WD) or both (DD) halves of the root system reduced g<sub>s</sub> in all genotypes significantly. Under the WD treatment, g<sub>s</sub> in Drysdale and Wyalkatchem decreased by an average of 35% compared to WW in ICGW-3262 and 20% in Drysdale. Under the DD treatment, g<sub>s</sub> declined by 70% in ICGW-3262 and by 50% in Wyalkatchem, Drysdale and ICGW-3119.

Transpiration rate was similar among genotypes within each treatment (WW mean=2.665 ± 0.3 mmol m<sup>-2</sup> s<sup>-1</sup>; P=0.21; Table 2). Withholding water significantly reduced transpiration rate in all genotypes (P<0.0001). It decreased to mean=-1.75 ± 0.3 mmol m<sup>-2</sup> s<sup>-1</sup> in WD and mean=-2.01 ± 0.03 mmol m<sup>-2</sup> s<sup>-1</sup> in DD treatments.

There was significant interaction effect of genotype x treatment for leaf net photosynthetic rate (P=0.01). Net photosynthetic rate was 10.4 μmol m<sup>-2</sup> s<sup>-1</sup> in all genotypes under the WW treatment (Table 2). Under the WD treatment, leaf net photosynthetic rate decreased by 70% in Drysdale, 40% in both Wyalkatchem and ICGW-3119, but only by 15% in ICGW-3262 (P<0.01). Leaf net photosynthesis in all genotypes was zero when watering was withheld from both halves of the root system (DD).

The genotypes Drysdale and ICGW-3262 had contrasting responses to water stress, but were expected to have comparable soil moisture use pattern due to their similar leaf area and root biomass. Hence ABA analysis was done only in Drysdale and ICGW-3262. There was a significant interaction of genotype x treatment for leaf ABA (P=0.001). Leaf ABA concentration increased two to three-fold when watering was withheld from one half (WD) or both halves (DD) of the root system in Drysdale (Fig. 3). In ICGW-3262, there was no increase in leaf ABA under the WD treatment, but it increased seven-fold under the DD treatment. Leaf ABA content in ICGW-3262 under the DD treatment was similar to WD and DD treatments in Drysdale.

3.3. Plant growth

At anthesis, Wyalkatchem had up to two-fold more tillers and green leaf area than the other three genotypes (P<0.0001; Fig. 4a and b). Shoot biomass also differed among genotypes at anthesis (P=0.004), where Wyalkatchem had the largest shoot biomass (Fig. 4c).

At second harvest, green leaf area of Wyalkatchem WW plants was largest (Table 3). Withholding water from one or both halves of the root system significantly reduced leaf area (P=0.001). Leaf area of plants under the WD treatment was 30% less in Wyalkatchem and 20% less in Drysdale compared with the leaf area of plants under the WW treatment (Table 3). Both ICGW lines under WD treatment had similar leaf areas to that of plants under the WW treatment. Leaves of plants under the DD treatment had begun to senesce and the leaf net photosynthetic rate was close to zero, so leaf area was not measured.

Tiller production increased in Drysdale and ICGW-3119 plants under the WW treatment after anthesis (Fig. 4a and Table 3). However, Wyalkatchem had 35% fewer tillers at second harvest compared with anthesis, whereas the number of tillers per plant in ICGW-3262 was unchanged. Withholding water from one (WD) or

Fig. 3. Mean leaf ABA concentration in the cultivar Drysdale and the line ICGW-3262 at 170-180 Cd and 100-110 Cd respectively after withholding water from one half (WD) or both halves of the split root system (DD) compared with both halves well-watered (WW). Error bars represent ±SEM. *P<0.05. Interaction for genotype x treatment was significant, P<0.001. LSD for the interaction was 2.006 μg g<sup>-1</sup> and different letters indicate significant differences between genotypes.
Table 3
Green leaf area and tiller number of four wheat genotypes grown in split pot. Plants were well-watered until maturity (WW) or watering was withheld from one (WD) or both (DD) halves of the split root system from anthesis. Second harvest was done at 190 Cd for ICW-3262 and 170 Cd for other three genotypes. Values presented are means (± 1). LSD values are at 95% level of significance. Interaction genotype x treatment was not significant (P=0.25).

<table>
<thead>
<tr>
<th></th>
<th>Green leaf area (m²)</th>
<th>Tiller number (per plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Second harvest</td>
<td>WD</td>
</tr>
<tr>
<td>ICW-3119</td>
<td>0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>Drysdale</td>
<td>0.14</td>
<td>0.11</td>
</tr>
<tr>
<td>Wyalkatchem</td>
<td>0.20</td>
<td>0.14</td>
</tr>
<tr>
<td>ICW-3262</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>LSD (genotype)</td>
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<td>2.3</td>
</tr>
<tr>
<td>LSD (treatment)</td>
<td>0.02</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Shoot biomass of all genotypes increased from anthesis to maturity when both halves of the split root system were well-watered (WW) (Fig. 4c, Table 4). Wyalkatchem had the lowest shoot biomass at final harvest of 55 g per plant, while the other three genotypes averaged 65 g per plant. Biomass accumulation at second harvest was not affected in ICW-3119 when water was withheld from one half of the root system (WD) while the other genotypes had significant reductions in biomass (P<0.001; Table 4). Withholding water from both halves of the split root system (DD) further reduced shoot biomass at second harvest in all genotypes. At final harvest, shoot biomass was significantly lower in all four genotypes (P<0.002), especially under the DD treatment which were reduced by 55% in Wyalkatchem to 70% in ICW-3262.

3.4. Root growth

Root biomass in each pot half was equal at anthesis, just before withholding water (P=0.46; Fig. 5a). Total root biomass (both pot halves combined) varied among genotypes. The line ICW-3119 had the smallest root biomass and Wyalkatchem the largest root biomass at anthesis and second harvest under WW conditions (Table 5). At second harvest, 170–190 Cd after watering was withheld from one half of the split root system (WD), total root biomass decreased in Drysdale, Wyalkatchem and ICW-3262, but not when watering was withheld from both halves of the root system (DD). Root biomass was unaffected by the watering treatments in ICW-3119 (Table 5). At final harvest, root biomass was not affected by watering treatment in Drysdale and ICW-3262, but was reduced in ICW-3119 and Wyalkatchem by similar amounts regardless of whether water was withheld from one or both sides of the pots (Table 5).

There were differences in total root length among the genotypes with Wyalkatchem having the longest (623 m) and Drysdale the shortest (291 m) (Table 6). There was a significant interaction between genotype and watering treatment (P=0.002) because of

Table 4
Shoot biomass of four wheat genotypes that were well-watered until maturity (WW) or withholding from one (WD) or both (DD) halves of the split root system from anthesis. The values are means of five replicates. LSD values are at 95% level of significance. Interaction genotype x treatment was not significant at second harvest (P=0.7) and final harvest (P=0.06).

<table>
<thead>
<tr>
<th></th>
<th>Shoot biomass (g per plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Second harvest</td>
</tr>
<tr>
<td></td>
<td>WW</td>
</tr>
<tr>
<td>ICW-3119</td>
<td>26.6</td>
</tr>
<tr>
<td>Drysdale</td>
<td>27.2</td>
</tr>
<tr>
<td>Wyalkatchem</td>
<td>26.9</td>
</tr>
<tr>
<td>ICW-3262</td>
<td>21.8</td>
</tr>
<tr>
<td>LSD (genotype)</td>
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<tr>
<td>LSD (treatment)</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Fig. 4. Mean tiller number (a), leaf area (b), and shoot biomass (c) per plant of four wheat genotypes at anthesis (205) and just before watering treatments were applied. Error bars represent ± SEM, n=5.
contrasting responses when water was withheld from one or both halves of the root system compared with when both halves of the root system were watered (WW). Total root length per plant in IGW-3119 was 23% greater than the other genotypes when water was withheld from one (WD) or both halves of the root system (DD). Root length in Drysdale under the WD treatment was similar to that under the WW treatment, but under the DD treatment it increased by 38%. In contrast, root length was decreased by 55% in Wyalkatchem and 43% in IGW-3262 in the WD treatment, but the reduction was negligible in the DD treatment (Table 6).

Specific root length (SRL) was similar among genotypes under the WW treatment except in Drysdale in which SRL was one-third lower than the other genotypes (Table 6). There was a significant interaction between genotype and watering treatment for SRL (P = 0.05) as Drysdale and IGW-3119 produced more fine roots in the treatments where water was withheld after anthesis, increasing root length per unit of weight by 32% and 18% respectively in both the WD and DD treatments, whereas SRL of Wyalkatchem and IGW-3262 was not affected by watering treatment (Table 6). SRL in each compartment varied with treatment, with more root length in the wet compartment (P = 0.017; Fig. 5b) in all genotypes.

### Table 5

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Second harvest (g/m²)</th>
<th>Maturity (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>WD</td>
<td>DD</td>
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<tr>
<td>IGW-3119</td>
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<td>LSD (treatment)</td>
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</tr>
<tr>
<td>LSD (genotype x treatment)</td>
<td>NS</td>
<td>0.77</td>
</tr>
</tbody>
</table>

### Table 6

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total root length (m)</th>
<th>Specific root length (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>WD</td>
<td>DD</td>
</tr>
<tr>
<td>IGW-3119</td>
<td>341</td>
<td>419</td>
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<tr>
<td>Drysdale</td>
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<td>308</td>
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<tr>
<td>Wyalkatchem</td>
<td>623</td>
<td>281</td>
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<tr>
<td>IGW-3262</td>
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<td>260</td>
</tr>
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<td>LSD (genotype)</td>
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<td>26</td>
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<td>LSD (treatment)</td>
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<td>22</td>
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<tr>
<td>LSD (genotype x treatment)</td>
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<td>45</td>
</tr>
</tbody>
</table>

3.5. Yield and yield components

There was a significant interaction between genotype x treatment on grain yield (P = 0.015). Wyalkatchem under the WW treatment had the lowest grain yield of about 20 g per plant, while Drysdale and IGW lines had similar yields of about 36 g per plant. Grain yield under the WD treatment decreased by 16% in IGW-3119, 17% in Wyalkatchem, 20% in Drysdale and 26% in IGW-3262 (Table 7; P < 0.05). Under the DD treatment, grain yield in all genotypes was only 10% under the WD treatment.

There was significant interaction effect of genotype x treatment for spikes per plant (P < 0.001). The number of spikes per plant varied from 14 in Drysdale to 21 in IGW-3262 (Table 7). The number of spikes in IGW-3262 decreased by 28% and 50%, respectively under the WD and DD treatments. The number of spikes per plant did not change in Wyalkatchem under both WD and DD treatments. The spike number in IGW-3119 and Drysdale was not affected under the WD treatment, but decreased by 21% and 26%, respectively under the DD treatment. Treatments in which water was withheld from one half (WD) and both halves of the root system (DD) did not change the number of spikelets per spike (P = 0.21; data not shown).

The number of grains per spike varied across the genotypes with Drysdale having the greatest number across all treatments followed by IGW-3262 (P < 0.0001; Table 7). The line IGW-3119 and cultivar Drysdale increased the number of grains per spike under the WD treatment compared with WW treatment by 63% (Table 7; P < 0.0001), whereas there was no change in IGW-3262 and Wyalkatchem. Under the DD treatment, there were 15–20% fewer grains per spike in all genotypes.

1000 grain weight under the WD treatment was highest in IGW-3119 (51 g) and lowest in IGW-3262 (40 g). Drysdale and Wyalkatchem had similar 1000 grain weights of 47 g under the WW treatment. Withholding water from one half of the root system (WD) reduced 1000 grain weight by 20% in IGW-3119, 12% in Drysdale and IGW-3262 and 7% in Wyalkatchem (P < 0.005). All genotypes had similar 1000 grain weights under the DD treatment. Wyalkatchem had lowest harvest index of 0.53 among WW plants.
Table 7

Yield components and yield of four wheat genotypes that were well-watered until maturity (WW) or watered withheld from anthesis from one (WD) or both (DD) halves of the split root system. Values are means (n=5) and LSD is at the 5% level of significance.

<table>
<thead>
<tr>
<th>Spikes per plant</th>
<th>Grain yield per spike</th>
<th>Thousand grain weight (g)</th>
<th>Grain yield (g) per plant</th>
<th>Harvest Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>WD</td>
<td>DD</td>
<td>WW</td>
<td>WD</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>IGW-3119</td>
<td>17.4</td>
<td>17.0</td>
<td>13.8</td>
<td>39.7</td>
</tr>
<tr>
<td>Drysdale</td>
<td>14.6</td>
<td>12.3</td>
<td>10.4</td>
<td>23.5</td>
</tr>
<tr>
<td>Wyalkitchem</td>
<td>20.5</td>
<td>14.6</td>
<td>10.2</td>
<td>46.8</td>
</tr>
<tr>
<td>LSD (genotype)</td>
<td>1.1</td>
<td>1.8</td>
<td>NS</td>
<td>2.3</td>
</tr>
<tr>
<td>LSD (genotype x treatment)</td>
<td>2.8</td>
<td>NS</td>
<td>NS</td>
<td>4.1</td>
</tr>
</tbody>
</table>

(P<0.003; Table 7). Harvest index increased in Drysdale and IGW-3119 under the WD treatment but did not change in Wyalkitchem and IGW-3262. Under the DD treatment, all genotypes had lower harvest index compared with WW treatment. Drysdale and IGW-3262 had higher harvest index under DD treatment (P<0.0001).

4. Discussion

Stomatal regulation in wheat in response to water deficit is reported to be closely related to increased leaf ABA concentration following a reduction in leaf water potential (Henson et al., 1989; Quarme and Lister, 1983). Contrasting responses in g, and leaf ABA concentration, as well as contrasting responses in Ws and increased leaf ABA (only measured in Drysdale). On the other hand, IGW lines reduced g, under the WD treatment and IGW lines increased Ws, indicating a higher percentage of leaf photosynthesis (Wp) or increase in leaf ABA. A lack of correlation between leaf ABA concentration and g, has been observed in maize (Tardieu et al., 1992; Zhang and Davies, 1990), despite ABA in the xylem sap correlating well with g, (Tardieu et al., 1992). The results with IGW-3262 indicate that high ABA concentration is not the main driver of decreased g, and probably does not reflect the ABA level acting on stomata in some wheat genotypes.

The contrasting responses in Ws when watering was withheld from one half of the split root system (WD) and the corresponding changes in stomata and leaf ABA indicate that the root system of the IGW lines detected the drying soil and produced a signal that triggered partial stomatal closure to prevent water loss and maintain leaf water balance. This root-derived signal could have been ABA (Hartung et al., 2002; Slovik et al., 1995), hydraulic (Christmann et al., 2007; Csomor, 2002), due to change in pH (Hartung et al., 1988; Sober et al., 2004) or change in the concentration of another phytohormone (Aharya and Assmann, 2009). Because water exchange and Ws were only measured at the second harvest, we do not know if Drysdale and Wyalkitchem also started to close stomata before any noticeable change in Ws or leaf ABA. However, other studies have observed that Drysdale tends to reduce transpiration rate only after soil water decreases considerably (Schappach and Sadok, 2012). Hence, Drysdale roots seem to be less sensitive to drying soil, but ABA increases in leaves in response to decreasing Ws (Hartung et al., 2002; Westgate et al., 1998), which ultimately caused closure of stomata. It therefore appears that stomatal responses in wheat under terminal drought occur in two stages: primarily in response to a root-derived signal and secondarily in response to a leaf-derived signal. Two-stage stomatal response to soil dryness is consistent with the observations in wheat by Xiong et al. (2013) and Du et al. (2013).

Wheat genotypes differed in their ability to maintain Ws when water was withheld from one half of the split root system (WD). Genotypic differences in the strategies to maintain leaf water status have been reported in species like grapes (Schultz, 2003), wherein isohydric varieties regulate stomata to maintain Ws and anisohydric varieties allow Ws to decline under soil water deficits (McDowell et al., 2008; Tardieu et al., 1992). Lack of soil moisture deficit makes it difficult to ensure that soil moisture status in all genotypes was similar, especially because the leaf area varied between genotypes. Genotypes reached anthesis at different days (Table 1) and hence, watering treatment also commenced on different days. However, vapour pressure deficit (VPD) during the period between anthesis and second harvest was 1.18 ± 0.034Pa for all genotypes, indicating similar driving force for transpiration. IGW-3262 reached permanent wilting stage by 190°Cd while in all other genotypes it was 170°Cd. Wyalkitchem plants might have dried the soil more quickly than IGW-3262 due to its larger leaf area and root biomass. But this does not appear to be the case with Drysdale since its leaf area and root biomass was similar to IGW-3262 at anthesis and when gas exchange measurements were made. Without the split root system and given the above details, IGW-3262 and Drysdale would be expected to have similar patterns in water use. Comparing the Ws in Drysdale and IGW-3262 WD plants with WW plants, IGW-3262 would be considered isohydric and the cultivar Drysdale as anisohydric. Contrasting isohydric and anisohydric behaviour has been observed previously in wheat genotypes, but under osmotic stress (Galil et al., 2013).

Genotypic difference in the maintenance of Ws in WD plants despite having similar leaf area, transpiration rate and VPD level indicates difference in their root level capability to supply water to meet the transpiration demand. The wet half of the Drysdale root system with access to sufficient water was unable to maintain the water status of the shoot, whereas IGW-3262 roots in the wet half of the root system supplied sufficient water to maintain shoot water status. Isohydric and anisohydric behaviour is regulated by stomatal control (McDowell et al., 2008) and/or hydraulic conductance (Schultz, 2003). Hence, we propose that isohydric genotypes adapt in response to root signals, maintaining leaf hydration possibly through root hydraulic conductance, which sustains stomatal regulation. However, as the soil drying intensifies, Ws declines inducing stomatal closure through increased leaf ABA synthesis. In contrast, Ws in anisohydric genotypes decreases either because their root system is less sensitive to soil water potential to initiate stomatal closure and/or their stomatal sensitivity to ABA probably depends on tissue water potential (Tardieu and Davies, 1992).

Drysdale increased SRL when watering was withheld from one half (WD) and both halves of the root system (DD) in response to decreasing soil moisture. An increase in root length with no increase in root biomass under the DD treatment suggests that the difference in SRL was due to production of thin roots and not due to root shrinkage (Huck et al., 1970). Some wheat genotypes are known to produce fine roots under water stress conditions (Manske and Vlek, 2002). Thin roots potentially increased the surface area per unit root volume, resulting in more effective water absorption
per unit mass. More thin roots (high SRL) produced in wet compartments than in dry compartments (Fig. 5) indicate a compensatory adjustment in all genotypes to supplement water supply to shoot. High specific root length and small root diameters are traits associated with drought resistance (Comas et al., 2013). However, thinner roots in wheat are also associated with lower root hydraulic conductivity because of narrower metaxylen vessels (Schoppach et al., 2014), which is considered beneficial for crops growing in environments with predominantly stored soil moisture (Richards and Passioura, 1993).

When water was withheld from one half of the split root system, reductions of 16–26% in grain yield occurred in all genotypes following 15–70% reduction in net photosynthetic rate, despite sufficient available water in the other half of the split pot. This suggests that the whole root system is needed after anthesis to fully meet the wheat plants demand for water and support grain production. This study also demonstrates that there is large genotypic variation in the impact of partial soil drying on wheat yield. The line ICGW-3262, which maintained leaf water status and gas exchange better than the other genotypes in response to drying signals had the highest yield reduction (25%) mainly due to producing fewer and smaller grains when watering was withheld from one half of the split root system. This is in disagreement with the findings of Westgate et al. (1996) where shoot water status had a positive effect on grain yield. Although the hydrated past part of ICGW-3262’s root system appears to be able to compensate better than other genotypes in maintaining leaf hydration under partial soil drying the superior gas exchange did not prevent reductions in shoot biomass to support grain development. In contrast, Drysdale’s grain yield was 6% greater than ICGW-3262 under partial soil water deficit despite Drysdale having the lowest instantaneous leaf photosynthetic rate. A large reduction in net photosynthetic rate compared with the reduction in gs, occurs in ageing wheat leaves (Atkinson et al., 1989). Interestingly, this does not translate to grain yield decline: possibly photosynthesis in ear parts of Drysdale contributed more towards grain development (Arau et al., 1991) or the plant partitioned more assimilates to grains (Blum, 1996). Harvest index was slightly higher in the WD than in the WW treatment in Drysdale indicating that this cultivar re-mobilised more assimilates to the grains. This is in agreement with the studies reported by Zhang et al. (2008). The higher leaf ABA concentration in Drysdale compared with ICGW-3262 under the WD treatment, might have also enhanced accumulation of water soluble carbohydrates in the stem and its remobilisation to grains (Travaglia et al., 2007; Yang et al., 2009). Wheat genotypes selected for high leaf ABA accumulation under drought had higher grain yield than low ABA accumulation types (Innes et al., 1984).

5. Conclusion

Wheat genotypes can have isohydric and anisohydric behaviour under water deficit. Stomata of isohydric genotypes seem to respond to an unknown signal, partially closing stomata. Under severe water deficit (DD treatment), increased leaf ABA is related to decreased leaf water potential. In contrast, stomatal response in anisohydric genotypes initiates with a drop in leaf water status and increase in leaf ABA concentration. Genotypic differences in synthesis of root ABA or other signals in response to water deficit is likely and needs to be considered in future studies. Some genotypes also responded morphologically to limited water supply by producing a deeper and denser root system. This physiological response, however, does not explain the difference in grain yield among genotypes when watering was withheld from one half of the split root system (WD).

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References
